Biochimica et Biophysica Acta 1817 (2012) 13–25

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

Photosystem II, a growing complex: Updates on newly discovered components and low molecular mass proteins ☆

Lan-Xin Shi [c], Michael Hall [a], Christiane Funk [a,b], Wolfgang P. Schröder [a,b,*]

[a] Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden
[b] Umeå Plant Science Centre (UPSC), Umeå University, SE-901 87 Umeå, Sweden
[c] Department of Plant Biology, University of California-Davis, One Shields Avenue, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 26 May 2011
Received in revised form 19 August 2011
Accepted 23 August 2011
Available online 30 August 2011

Keywords:
Psb
Photosynthesis
Arabidopsis
Oxygen evolution

ABSTRACT

Photosystem II is a unique complex capable of absorbing light and splitting water. The complex has been thoroughly studied and to date there are more than 40 proteins identified, which bind to the complex either stably or transiently. Another special feature of this complex is the unusually high content of low molecular mass proteins that represent more than half of the proteins. In this review we summarize the recent findings on the low molecular mass proteins (<15 kDa) and present an overview of the newly identified components as well. We have also performed co-expression analysis of the genes encoding PSII proteins to see if the low molecular mass proteins form a specific sub-group within the Photosystem II complex. Interestingly we found that the chloroplast-localized genes encoding PSII proteins display a different response to environmental and stress conditions compared to the nuclear localized genes. This article is part of a Special Issue entitled: Photosystem II.

1. Introduction

Photosynthesis is a fundamental process for life on earth whereby sunlight is converted into chemical energy and carbon dioxide is bound into carbohydrates used as a food and energy source by heterotrophs such as humans. Additionally, oxygen is released as a by-product in the first step of the photosynthetic process of vascular plants, algae, and cyanobacteria. A protein super-complex referred to as Photosystem II (PSII) absorbs sunlight and initiates electron transport. This protein complex has been studied extensively — an internet search on the words 'PSII' and 'plants' results in more than 300,000 hits and PubMed reveals almost 200 hits covering only the years 2010 and 2011. Structural data of the PSII core complex from various bacteria have emerged, *e.g.* the crystal structure of PSII from *Thermosynechococcus elongatus* has been resolved at 3.8–2.9 Å [1–5] and recently from *T. vulcanus* at 1.9 Å [6]. This has enabled a detailed analysis of the water splitting Mn-cluster, chlorophylls, carotenes, lipids and quinones within the complex. However, still awaiting is a high resolution structural analysis of PSII from higher plants.

Based on electron microscopical and imaging analyses it is evident that in the thylakoid membrane PSII arranges to super-complexes that have a molecular mass of almost 1100 kDa (see review Dekker and Boekema [7]). These supercomplexes are formed by PSII-dimers and the LHC complexes. Each monomeric PSII complex contains almost 40 proteins, which are either permanently bound to the complex or transiently associated with the PSII complex. Only 10 years ago PSII was thought to consist of just 20 subunits [8]. Five years later the number of PSII associated components had increased to 30 [9]. Surprisingly, today new components belonging to the PSII complex are still being identified and reported. This is accomplished by the use of more sensitive techniques, such as liquid chromatography mass spectrometry (LC–MS) or SDS-PAGE in combination with mass spectrometry (MS) techniques [10,11]. Several of these proteins are expressed or bound to PSII only during special situations such as stress, degradation, or assembly.

A special feature of the PSII complex is the presence of many low molecular mass proteins (Fig. 1). More than half of the proteins in the complex have molecular masses below 15 kDa, several of which weigh only roughly 5 kDa which corresponds to 40–60 amino acids. These peptides are just long enough to span the hydrophobic membrane with a single α-helix. Due to their small size and hydrophobicity it is difficult to generate specific antibodies, which are important for biochemical studies. Also techniques such as mass spectrometry only give limited success, as the low molecular mass proteins contain no or only very few cleavage sites. In this review we will focus on the latest published data on low molecular mass proteins associated with PSII. We will describe recently identified "new" PSII proteins and discuss their detection, structure, and suggested function. We will also address the question of whether the low molecular mass proteins

Abbreviations: PSII, Photosystem II; Psb, protein subunit of Photosystem II; cyt b_{559}, cytochrome b_{559}

☆ This article is part of a Special Issue entitled: Photosystem II.

* Corresponding author at: Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden. Tel.: +46 907866974; fax: +46 907867655.
E-mail address: wolfgang.schroder@chem.umu.se (W.P. Schröder).

0005-2728/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2011.08.008

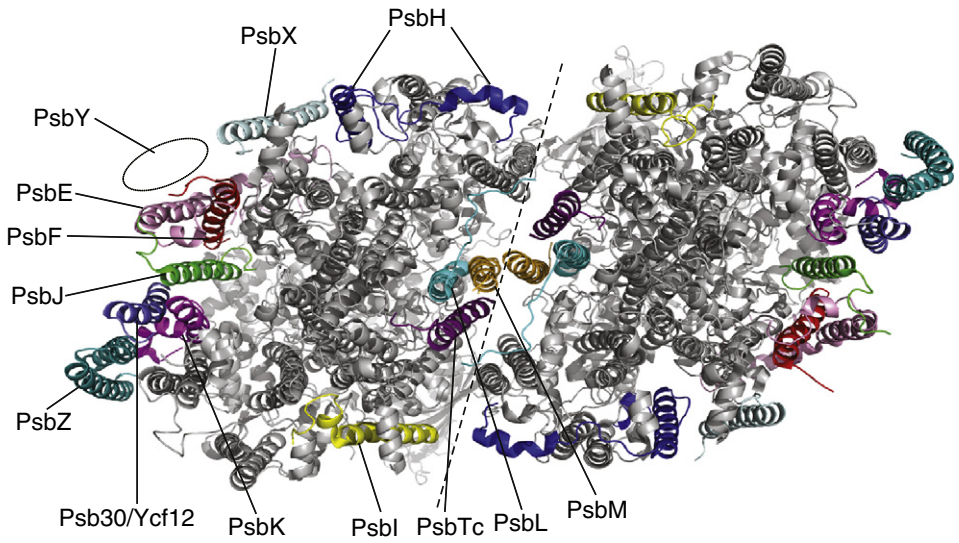

Fig. 1. Structure of homodimeric PSII complex from *T. vulcanus* (PDB ID: 3ARC[6]) generated by PyMol molecular graphics program (v1.3, Schrödinger, LLC). Overview of PSII from the cytoplasmic side. The monomer–monomer interface is indicated by a black dashed line. Low molecular mass proteins: PsbE (pink), PsbF (red), PsbH (blue), PsbI (yellow), PsbJ (green), PsbK (magenta), PsbL (cyan), PsbM (bright orange), Psb30/Ycf12 (slate), PsbX (pale cyan) and PsbZ (teal) are labeled in the left-side monomer. The position of PsbY that was missing in the crystal is marked with a circle according to a previous model [2]. PSII core subunits, D1, D2, CP47 and CP43 are colored in gray. The oxygen evolving complex, PsbO, PsbU and PsbV are also in gray.

are a specific "homogenous" sub-group in PSII. For additional information on low molecular mass proteins, *e.g.* sequence information and history of discovery, we would like to refer the reader to our previous review on this topic [9].

2. Updates on low molecular mass proteins

Since 2004 (see Shi and Schröder [9]) roughly 20 new publications dealing with low molecular mass proteins of PSII were released. In order to discuss the localization of these proteins within PSII we assume similar localization and orientation of bacterial and eukaryotic orthologues. As mentioned earlier, high-resolution structural data derived from eukaryotic PSII, enabling the identification of low molecular mass proteins in higher plants, are not available.

2.1. PsbI

PsbI (the 4.8 kDa polypeptide) is tightly associated with PSII reaction center complexes (RC). Studies on the PSII crystal structure reveal that it is located at the periphery of two monomeric cores [1,2,4,6] and may serve as a linker, connecting the core complex to the minor antenna protein CP29 (Lhcb4) and the more distal LHCII trimer [7]. Its close neighbors within the PSII core are the reaction center protein D1 and the core antenna protein CP43 [1,2,4,6]. PsbI knock-out mutants in the eukaryotic unicellular green alga *Chlamydomonas reinhardtii*[12] and the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis* 6803) [13] were produced and characterized in 1995. The eukaryotic phenotype was more severe than in the cyanobacterium [12,13]. Homoplastomic *psb*I knock-out in a higher plant, tobacco, was generated using a transplastomic approach [14]. Although the *psb*I mutants from all three species grew photoautotrophically, the eukaryotic mutants were highly sensitive to high-intensity light [12–14].

Deletion of *psb*I in tobacco caused decreased levels of the PSII core components, PSII dimer complexes and super-complexes. Notably, substantial amounts of *de novo* synthesized PSII dimer and PSII–LHCII complexes were detected in the tobacco *psb*I mutant, which led the authors to the suggestion that PsbI plays an important role in stabilization of dimer and super-complexes rather than in an efficient assembly process of the complexes [14]. Other consequences of *Psb*I inactivation included destabilization of the Q_A midpoint potential, reduction of energy transfer to the PSII reaction center and PSII/PSI ratio, abolishment of PSII core protein phosphorylation, and reverse regulation of LHCII phosphorylation compared to wild type [14]. The lack of PsbI altered the PSII structure, presumably by loosening the connection between the two monomers and between the inner and outer antennae. It is concluded that PsbI has a dual function; it is required for proper functioning of PSII and also for stabilization of PSII dimeric complexes and super-complexes in tobacco [14].

Recent studies in the cyanobacterium *Synechocystis* 6803 demonstrated that PsbI is involved in two stages of PSII assembly; i) binding to newly synthesized D1 protein at an early stage of the *de novo* PSII assembly and ii) stabilizing the association of CP43 to the PSII core complexes [87].

Deletion of the *psbI* gene in the thermophilic cyanobacterium *T. elongatus* decreased the amount of PSII dimers [38]. PsbI therefore may take a part in the assembly of PSII dimer complexes. Once the dimers are formed, PsbI is not crucial for stabilizing the PSII dimer complexes.

The different roles of PsbI suggested in the aforementioned studies in eukaryotic and prokaryotic species may reflect differences in the molecular micro-environment surrounding PsbI that evolved over time. In eukaryotes, PsbI is a linker connecting the inner antenna (CP43) with the outer antenna (CP29 and possibly LHCII trimer), while in cyanobacteria the outer antenna consist of extrinsic phycobilisomes, PsbI therefore is localized at the boundary between PSII and lipids of the thylakoid membrane.

2.2. PsbK

Removal of PsbK did not influence photoautotrophic growth of the mutant in cyanobacteria [15,16]. However, biochemical thylakoid preparations from *psb*K resulted in a loss of PsbZ, and in isolated PSII complexes from the mutant, both PsbZ and Psb30 (Ycf12) were lost [15]. These data support the location of PsbK in PSII crystals [2,6]; it was assigned, together with PsbZ and Psb30/Ycf12, to be located near CP43 in the peripheral region of the PSII dimer. *Chlamydomonas psb*K knock-outs, in contrast to the cyanobacterial mutants, were unable to grow photoautotrophically [17] and the amount of

PSII complexes decreased to less than 10% of that in wild type due to instability and faster degradation.

2.3. PsbJ and PsbL

In most species performing oxygenic photosynthesis, PsbJ and PsbL are encoded in the *psbEFLJ* operon together with PsbE and PsbF [18]. The two subunits of cyt b_{559}, PsbE and PsbF, are low molecular mass proteins that are essential for the structure and function of PSII [19–23]. In the PSII crystal structure, PsbJ is assigned close to PsbE and PsbF; together, these three subunits seem to form an entrance of a putative channel for quinone diffusion toward the Q_B site in D1 [2,24]. In both cyanobateria and higher plants, deletion of PsbJ generated much longer lifetime of reduced Q_A [25,26] compared to wild type. It was concluded that PsbJ plays a role in regulation of forward electron flow from reduced Q_A to the plastoquinone pool [26]. Deletion of PsbJ in *Synechocystis* 6803 decreased oxygen evolution compared to wild type [25,27]. A tobacco *psb*J deletion mutant [22,23,25,26,28] could not grow photoautotrophically and was highly sensitive to light [22,28]. The mutant only possesses residual PSII activities in young leaves. Biochemical isolated PSII from this mutant was dimeric with reduced amounts of PsbQ, and PsbP was lacking in these preparations [22,23]. Therefore PsbJ seems to be important for the assembly of the extrinsic protein PsbP into PSII complexes [23,28].

PsbL along with PsbM and PsbTc are located at the monomer–monomer interface of PSII complexes in proximity to the Q_A site in the reaction center protein D2 [1,2,4,6]. Inactivation of PsbL resulted in loss of photoautotrophical growth and PSII activity in both *Synechocystis* 6803 and tobacco [22,29]. The tobacco mutant was quickly bleached in light [22]. In contrast to *psb*J, *psb*L did not form stable PSII dimer complexes; instead, the monomeric form was predominantly detected in partially solubilized thylakoid membranes [22,23]. In addition, CP43 was only labile associated with these monomers, resulting in loss of PsbP and PsbQ [23]. The last four C-terminal amino acids of PsbL seem to be essential for this protein to assemble to PSII complexes [30]. It has been reported that PsbL is involved in the restoration of Q_A activity on the acceptor side [31,32] and in oxidation of TyrZ by $P680^+$ on the donor side [33]. Based on its presumed location within PSII and on further characterization of *psb*L, this subunit is likely to act on the acceptor side in preventing reduction of PSII by back electron flow from plastoquinol [2,4,6,26].

2.4. PsbM

The PsbM protein has been detected in cyanobacteria, Chlamydomonas and in higher plants such as *Arabidopsis*. Biochemical and biophysical analyses of a *psb*M knock-out mutant in tobacco did not reveal any differences in formation of PSII–LHCII supercomplexes [36], but interestingly PSII was more light sensitive and the phosphorylation of D1 and D2 proteins was reduced. Furthermore, LHCII was found to be reversely regulated due to accumulation of reduced plastoquinone in the dark and reduced PSII electron transport in the light. The Q_B site of PSII was altered in the absence of the PsbM protein, and thus the protein is primarily involved in the electron flow within and outwards of PSII [36].

Somewhat contrary to the results from tobacco in *Synechocystis* 6803 the backward electron flow was not affected [37]. However, the mutant also displayed light sensitivity and rapid photoinactivation. Additionally, *psb*M mutants contained less PSII centers (28%), suggesting an important role for PsbM in PSII assembly.

The PsbM protein has been shown to be located in the center of the PSII dimer [2,6]. Two PsbM proteins from each monomer are suggested to be connected via a leucine zipper to hold the two PSII subunits together. In this manner the PsbM protein would be crucial for dimer formation. Comparing the crystal structure of PSII dimers from mutants lacking PsbM [38] and from wild-type confirmed the central location of PsbM as being in-between the two PSII monomers and also revealed the detailed crystal structure of the PsbM protein. The mutant had decreased amount of PSII dimers and a corresponding increase in monomeric PSII. Nevertheless, a significant amount of PSII dimers were detected in the PsbM mutant, indicating that PsbM is not the only protein responsible for dimer formation.

2.5. PsbR

PsbR is also known as the 10 kDa PSII polypeptide. This intrinsic protein is nuclear encoded and only present in green algae and higher plants, hence absent in the crystal structure of cyanobacterial PSII. Biochemical studies and sequence analyses show that PsbR has a transmembrane span and the majority of the protein is located on the lumenal side of the thylakoid membrane [34] (also see review [9] and references therein).

In an *Arabidopsis* T-DNA insertion line [35,143] the rate of oxygen evolution was reduced, especially under low light growth conditions. Both donor and acceptor sides of PSII are modified in this *psbR* mutant [143,144]. Strikingly, levels of PsbP and PsbQ were strongly decreased in this mutant compared to wild type [35,144], likely due to proteolytic degradation because of their retarded assembly to PSII core complexes. The defects of the *psbR* mutant could be near fully recovered by expression of a C-terminal His6-tagged PsbR [144]. Therefore, PsbR is suggested to be the docking protein for PsbP (and indirectly for PsbQ) to form the oxygen evolving complex together with PsbO [35,144]. Furthermore, effective assembly of PsbR in PSII complexes is dependent on PsbJ [35].

2.6. PsbTc

PsbTc, the chloroplast encoded intrinsic polypeptide is conserved from cyanobacteria through higher plants. It is important to distinguish PsbTc from PsbTn, which is a soluble, nuclear encoded protein. Thus we encourage the use of the two subscriptions for clarity. In the PSII crystal structure, PsbTc is located at the interface of the two monomers, close to PsbM and PsbL [1,2,4,6].

The PsbTc gene has been inactivated in cyanobacteria [37,43], green algae [39–41], and in tobacco [42]. The *psb*Tc mutant in *Synechocystis* 6803 showed a slower doubling time and 40% reduction in PSII assembly and oxygen evolution compared to wild-type [37]. Additionally, Q_A^- reoxidation was slowed down indicating a role of PsbTc on the acceptor side. This finding was supported by accelerated photodamage of PSII activity seen in these mutants. PsbTc seems closely connected to PsbM in localization and function as only monomeric PSII was detected in the *psbM psbTc* double mutant. Somewhat contradictory, the *psb*Tc knock-out mutants obtained in *Thermosynechococcus elongates* displayed no changes in photoautotrophical growth rate or oxygen evolution. However, dimeric PSII was dramatically reduced [43]. These authors found that in *Thermosynechococcus* PsbTc is critical for binding PsbM, while PsbM does not appear to be necessary for the binding of PsbTc. These data are supported by the PSII crystal structure (Fig. 1), PsbM seems to be closely located to PsbTc, but also to PsbL.

In *Chlamydomonas reinhardtii* PsbTc has been suggested to be required for efficient posttranslational repair of photodamaged PSII [39]. A more detailed study showed that PsbTc is important for Q_A binding as well as for the reactivation of Q_A after photoinhibition [40]. Both PsbTc and also PsbL are closely located to Q_A (Fig. 1, [6]), thus disturbance of either of these proteins could affect the stability of Q_A and its reactivation. A *psb*Tc mutant in the Chlamydomonas *y-1* background [41], which is used for studies on biogenesis of chlorophyll-complexes in the thylakoid membrane, synthesized PSII proteins at normal levels. Thus the effects observed in the *psbTc* mutant were not due to changes in protein synthesis, but instead PsbTc seems to be important in the

early assembly steps of PSII. Thus, PsbTc is not only important for the repair, but also the assembly of PSII.

Recently a PsbTc mutant was created and analyzed from tobacco [42] which displayed normal photoautotrophic growth, no visual phenotype and the amount of PSII proteins was not affected. Notably, electron transport, LHCII assembly, and PSII stability was strongly influenced and furthermore this tobacco PsbTc mutant shows delayed recovery from photoinhibition.

2.7. PsbW

This 6.1 kDa nuclear encoded protein was originally identified in various biochemical preparations of higher plant PSII [44,45], and thus was suggested to be a part of it. The corresponding gene is absent in cyanobacteria. Comparison of wild type plants with a *psb*W antisense mutant in *Arabidopsis* revealed that the protein is important for stabilization of the PSII dimer [46] and later the protein was found to be imported into dimeric PSII-supercomplexes [47], also referred to as PSII–LHCII-supercomplexes [48] Data obtained by Rokka et al. [49], however, suggested the PsbW protein to be closely localized to LHCII, as it was found to assemble to PSII concomitantly with the Lhcb proteins. These results were also supported by MS data [10]. The contradictory results were unified by the work of Garcia-Cerdan et al. [50], comparing PsbW antisense and knock-out plants using mild separation methods such as native gel electrophoresis and sucrose gradient centrifugation. In the absence of the PsbW protein no PSII–LHCII supercomplexes were formed. Additionally, electron microscopy analysis showed that the semicrystalline macro-domains of PSII–LHCII supercomplexes visible in wild type thylakoid membranes, could not be observed in the knock-out mutant. Changes in the macro-organization led to a decrease in phosphorylation of PSII proteins, a modified redox state of the plastoquinone pool, and changes in state transition. Based on these results PsbW should be located close to the minor antennae complexes in PSII [50]. In light of the fact that PsbW is not present in cyanobacteria, we can suggest that super-complex formation of PSII in large crystalline arrays is more important in higher plants. So far no suggestions for the localization of PsbW within PSII are possible, however, based on its proposed function it very likely could be located at the site X identified by Dekker and Boekema [7], which is situated close to the minor antenna protein CP26. The negatively charged N-terminus of PsbW could in this position have a crucial function for super-complex formation.

2.8. PsbX

The nuclear encoded PsbX protein has a molecular mass of 4.1 kDa and is found in PSII complexes of cyanobacteria and plants [9]. It is shown that the protein is not present in PSII reaction center complexes; instead in the PSII core preparations [51]. Cross-linking combined with immunoblotting demonstrated that the closest neighbor of the protein is cyt b_{559} [51], which is confirmed by structural studies recently [2,6]. Data on *psbX* deletion mutants in the cyanobacteria *Synechocystis* 6803 [52] and *T. elongatus* [53] indicated that PsbX is involved in quinone turnover at the Q_B site of the PSII reaction center. The mutants had a reduced amount of functional PSII. Data on knock-out mutants of higher plants have not been reported so far, however, in *Arabidopsis psb*X antisense plants containing less than 10% of the protein, the amount of functional PSII was also reduced by 30–40% [54]. These plants did not show any obvious phenotype when grown under normal conditions, but exhibited decreased phosphorylation of their PSII core proteins and LHCII when exposed to different light intensities. Therefore the redox state in the thylakoid membranes seems to be changed in the absence of the PsbX protein, and the plastoquinone pool is in a more oxidized state [54].

The cyanobacterial crystal structure obtained by Loll et al. [4] did not assign a specific location for PsbX, except the possible position at one of the three "unknown helices", denoted X1, *X*2 and X3. The structure reported by Kemiya and Shen [3] suggested a close location of PsbX to cyt b_{559}, which would correspond to the X3 helix. The most recent crystal structure with a resolution of 2.9 Å and 1.9 Å [2,6] (also see Fig. 1) confirm this suggested location for PsbX, which is in line with the biochemical data as mentioned above, as well [51]. The X1 helix was shown to correspond to Psb30/Ycf12 while *X*2 to PsbY [2].

2.9. PsbY

Crystallographic analyses of PSII derived from *T. elongates* annotated three unidentified helices denoted X1, *X*2 and X3 [4]. *X*2 was missing in the structure obtained by [1] and also in PSII crystals of a *psbY* deletion mutant in *T. elongatus* [55]. Even in the recent 1.9 Å crystal structure of PSII from *Thermosynechococcus vulcanus* the PsbY protein was not observed and was suggested to be lost during isolation, which probably reflects its peripheral location [6]. Nevertheless, PsbY is shown to be located at the periphery of PSII, in the vicinity of the two α-helixes of cyt b_{559} in the structural map at 2.9 Å resolution [2]. The position of PsbY indicated in this crystal structure is equivalent to *X*2 in the 3.0 Å structure [4]. More detailed analysis of the crystal structure containing PsbY revealed that the long hydrophilic loop of the protein protrudes into the stromal side of the membrane, while its N-terminus is located directly at the luminal membrane surface. These results support the fact that the PsbY protein is not involved in PSII water splitting as originally suggested [56]; due to its more distant location it cannot provide any ligands for the Mn_4Ca-cluster. To clarify the function of PsbY we have obtained *Arabidopsis* knock-out mutants. The observed phenotypic changes at cyt b_{559} (von Sydow, Garcia-Cerdan, Meurer, Schröder, unpublished results) are in good agreement with the assigned location of PsbY in the structure model [2].

2.10. PsbZ

The PsbZ protein is the only small subunit with two transmembrane helices; therefore both N- and C-terminus are located on the same side of the membrane [24,57]. Based on purified and crystallized PSII from *psbZ* deletion mutants from *Themosynechococcus*[57] the location of this protein was shown to be on the outside of the PSII complex, close to the two small proteins PsbK and Psb30, and to CP43 of the PSII core. Interestingly, PsbZ seems to cap the two other small proteins, as both PsbK and Psb30 are lost during isolation of PSII from the *psbZ* mutants. Meanwhile PsbZ is not lost during PSII isolation from the *psb30* mutant [58]. These findings led to the suggestion that the order of assembly of the proteins is Psb30, PsbK and last PsbZ [57,58]. Mutants lacking the PsbZ protein in *Synechocystis* 6803 have been created and analyzed [59]. This is of special interest because in higher plants it has been suggested that the PsbZ protein could be involved in the integration and functioning of CP26, a minor antenna found only in higher plants [60]. However, biochemical and biophysical analysis in *Synechocystis* 6803 did not reveal any significant changes under standard conditions. At low light, the cells displayed slower growth than wild type and in the presence of glucose lack of growth was observed. It therefore was suggested that PsbZ could play a role in protection against photoinhibition in a similar manner as suggested for PsbK [61]. This is contradictory to the finding that *Thermosynechococcus* PsbZ knock-out mutants grew faster under high light compared to wild-type [58]. The function of PsbZ in cyanobacteria might also be different to the one in higher plants, where the photoprotective role of the PsbZ protein seen in cyanobacteria might have been taken up by the minor antenna complex during evolution.

3. Newly identified PSII components

The following section reviews recent progress on six newly identified proteins belonging to PSII, named Psb27 through Psb32 as well as

on the stress-induced light-harvesting like proteins associated with PSII. The features of these proteins are summarized in Tab. 1.

3.1. Psb27

The Psb27 protein was first designated as PsbZ, but, because the name already referred to another protein, later renamed Psb27 [62]. Alternative names of Psb27 are the PSII 11 kDa protein (Cyanobase) and Low PSII Accumulation 19 (LPA19) [63].

Psb27 was initially identified in PSII complexes purified from *Synechocystis* 6803 [62], [64] and *T.vulcanus*[65]. It was also detected in PSII complexes from the red alga *Galdieria sulphuraria* using top-down high-resolution MS [66] and in PSII core complexes isolated from tobacco [67]. Noteworthy, *Psb*27 is found in almost all sequenced genomes of organisms performing oxygenic photosynthesis so far, except *Gloeobacter violaceus* PCC 7421, a cyanobacterium that lacks thylakoids [68].

Sequence analysis indicates that Psb27 is soluble without any transmembrane domain [69–71]. It has been detected in the *Arabidopsis* lumen proteome [72–74] and the cyanobacterial thylakoid proteome [75]. *Synechocystis* Psb27 (Slr1645) contains 134 amino acids including an N-terminal signal peptide (24 amino acids). The molecular weights of the precursor and mature protein are 14.8 and 12.3 kDa, respectively. In *Arabidopsis* two Psb27 proteins have been detected, designated as Psb27-H1 (AT1G03600) and Psb27-H2 (AT1G05385) [63]. The two precursor proteins consist of 174 and 199 amino acids with molecular masses of 18.8 and 22.3 kDa, respectively. After cleavage of the stromal and thylakoid targeting peptides, the mature forms of the two proteins are 11.8 and 15.1 kDa, respectively. The two mature proteins share only 23% identical sequence. It is not clear at the present whether or not these two proteins have distinct roles. The *Arabidopsis* Psb27-H1 is predicted to have a Tat motif in its signal peptide, supporting its lumenal location [70,72,73]. The structure of Psb27 in solution has been determined by using NMR spectroscopy [76–79]. The protein possesses a single domain arranged in a right-handed four-helix bundle with an up–down–up–down topology. The core structure is stabilized by hydrophobic interactions between the four amphipathic helices [76,78] and the distribution of electrostatic potential on the surface is dipolar. Psb27 is predicted to bind to a niche formed mainly by CP47 and D2 proteins of PSII [76,77]. The same area in the PSII crystal structure is shown for binding PsbO [2], which agrees with the biochemical findings suggesting that Psb27 prevents binding of extrinsic lumenal proteins onto pre-assembled PSII complexes [80] (see below).

Cyanobacterial Psb27 is a bacterial lipoprotein tightly associated with the thylakoid membrane through its N-terminal lipid modification; therefore it cannot be removed from monomeric PSII complexes by washes with solutions containing either high salt concentration or high pH [65,71]. In contrast, the *Arabidopsis* Psb27-H1 and -H2 do not have such a lipobox motif. Psb27-H2 (also known as Lpa19) can be removed with 1 M $CaCl_2$ or 6 M urea from sonicated thylakoid membranes, supporting its peripheral association with the membranes [63].

Functional studies on Psb27 have been conducted in both cyanobacteria and *Arabidopsis*. Psb27 was preferentially found in the wild-type monomer of PSII from *Synechocystis*[81]. In the *Synechocystis* mutant lacking CtpA, a protease that produces mature D1 protein by removing its C-terminal extension, isolated PSII does neither contain manganese nor any of the extrinsic lumenal proteins PsbO, PsbU and PsbV. Strikingly, Psb27 is more abundant in these PSII complexes [69]. It therefore might transiently associate with PSII to facilitate the latter's assembly [69]. Similar results were obtained in PSII complexes from *T. elongatus* [71]. Rögner and his colleagues have purified and characterized four PSII complexes from *T. elongatus*, low and high molecular weights of monomer and dimer PSII, respectively [71]. The low molecular weight PSII monomer (PSIImLow) are inactive because they have lost manganese and the extrinsic proteins, PsbO, PsbU and PsbV. Instead, they contain an extra protein, Psb27. Binding of Psb27 to the lumenal side of PSII seems to block the reconstitution of the three extrinsic proteins onto the complex. Lacking an active Mn_4Ca cluster, PSIImLow complexes have a perturbed donor side and forward electron transfer from the primary quinone Q_A is impaired [82]. It is concluded that the inactive Psb27-containing PSII monomers are PSII intermediates that are involved in PSII biogenesis, especially in the repair of photodamaged PSII [71]. In *Synechocystis* 6803, the step of PSII assembly at which Psb27 is functioning has been precisely defined [80]. Deletion of Psb27 did not affect photoautotrophic growth or oxygen-evolving activities in the mutant when grown in nutrient-rich medium. Upon high light illumination, the PSII recovery from photoinhibition in the mutant was slower than in wild type. Psb27, especially under high light, therefore should facilitate the assembly of the manganese cluster. Furthermore, it was demonstrated that Psb27 serves as a structural substitute for the extrinsic lumenal proteins of PSII during PSII assembly/disassembly. The transient binding of Psb27 to PSII blocks association of PsbO, and presumably also of the other extrinsic lumenal proteins to the Mn_4Ca cluster-lacking PSII during PSII biogenesis, namely repairing photodamaged PSII [80]. Characterization of other *Synechocystis* mutants lacking PsbM, PsbTc or Psb27, and double mutants lacking two of these proteins suggests that Psb27 is important for PSII biogenesis in the *psbM* mutant and for recovery of photodamaged PSII in the *psbTc* mutant [37].

Characterization of the *psb27* (At1g03600, Psb27-H1) T-DNA insertion mutant in *Arabidopsis* indicated that deletion of Psb27 does not significantly affect function and protein composition of PSII under normal growth conditions [70]. The degradation rate of photodamaged D1 in the mutant was comparable with that in wild type. However, the mutant was more susceptible to high light, in terms of decreased PSII activities and D1 protein levels than wild type leading to the conclusion that the protein is required for efficient recovery of damaged PSII from photoinhibition [70].

The Psb27-H2 (At1g05385, Lpa19) mutant, *lpa*19, displays a high chlorophyll fluorescence phenotype [63]. Leaves of the mutant are pale green and accumulate less PSII components than wild type. The maximum efficiency of PSII was much lower in the mutant and it is more susceptible to high light. Also the C-terminal processing of D1 precursor protein is impaired in this mutant. The interaction between Psb27-H2 and the D1 protein was supported by using yeast two-hybrid system and co-immunoprecipitation.

Taken together, Psb27 seems to be transiently associated with PSII monomeric complexes at the lumenal side during PSII biogenesis. It is proposed to facilitate the C-terminal processing of D1, assembly of Mn_4Ca cluster, and to prevent the extrinsic proteins from binding to the pre-assembled PSII. The function of Psb27 is more pronounced under high light, because faster PSII turnover takes place.

3.2. Psb28

The Psb28 protein is encoded by *sll1398* in *Synechocystis* 6803; *slr1739* has also been annotated as Psb28-2, however, this gene only has low similarity with *psb*28 (18% identical). In eukaryotes Psb28 can be either plastid (*ycf79*) or nuclear encoded [83]. Interestingly, in *Thalassiosira pseudonana*, a centric diatom, there are two similar *Psb28* genes, one is located in the nucleus, the other one in the plastid [84]. It is demonstrated that both genes in *T. pseudonana* are transcribed and the nuclear gene product fused to the yellow fluorescent protein is indeed targeted into the complex plastids directed by the N-terminal bipartite presequence containing signal peptide and transit peptide [85]. In contrast, there is only one *Psb28* gene in the plastid genome of another diatom *Phaeodactylum tricornutum*[83]. The authors propose that copying of this *Psb28* gene into the *T. pseudonana* nuclear genome may be a recent event [83] giving an example that gene transfer from the endosymbiont's genome to its host nuclear genome is still an ongoing process [83,85]. In *Arabidopsis*, Psb28 is the

product of a nuclear gene, At4g28660. Because its apparent molecular weight is 13 kDa, it also is known as Psb13 [87, also at Cyanobase (http://genome.kazusa.or.jp/cyanobase/Synechocystis)].

The protein belongs to Psb28 family (Pfam Id: PF03912) comprising 48 members [86]. The molecular weights of the protein in *Synechocystis* and *Arabidopsis* are 12.6 and 15.1 kDa containing 112 and 134 amino acid residues, respectively. The solution NMR structure of *Synechocystis* Psb28 has been recently reported [86]. The structure possesses two short helices, one long α-helix, two anti-parallel β-sheets and nine loop regions. In addition, nine solvent accessible cavities are identified, among which two contain highly conserved amino acid residues, suggesting that these two cavities may be important in interacting with PSII core subunits [86].

Psb28 was found to be associated with PSII in *Synechocystis*[62,64]. It was also detected in the PSII complex from the red alga, *Galdieria sulphuraria*[66]. The amount of protein bound to PSII complexes is sub-stoichiometric and it can be easily removed by $CaCl_2$ wash [62,87]. It is completely washed off the membranes by 0.1 M sodium carbonate and sodium hydroxide, and is digested by trypsin indicating its peripheral location on the thylakoid membranes [87]. Most Psb28 is present as free, unassembled protein, only a portion of the protein is associated with PSII core complexes lacking inner antenna CP43 (referred as RC47); it is absent in PSII dimers as analyzed by two dimensional blue-native/SDS-PAGE and immunoblotting. Moreover, this protein is also associated with unassembled CP47; in a CP47 knockout mutant, Psb28 was undetectable [87].

A Psb28 deletion mutant generated in *Synechocystis*[87] showed slower growth rates and lower chlorophyll levels. The absence of Psb28 affected PSI levels, *e.g.* the PsaA/PsaB heterodimer. Although deletion of this protein did not affect the function of fully assembled PSII complexes, it resulted in slightly increased D1 turnover and more efficient repair of PSII. Two-dimensional blue-native/SDS-PAGE analysis demonstrated that in the mutant, reaction center complexes and unassembled D1 accumulate, whereas unassembled CP47 is absent, suggesting that PSII assembly is limited by availability of CP47. Reduction in unassembled CP47 and PsaA/PsaB, all Chl containing proteins, is caused by less efficient Chl synthesis in this mutant.

Another mutant of *Synechocystis* 6803, unable to synthesize phosphatidylglycerol (PG) and therefore in need of PG supply to the growth medium, accumulated Psb28 in its cells, most of which is bound to monomeric PSII complexes [81]. Accordingly, the authors suggested that this extrinsic subunit may be involved in PSII assembly [81]. Psb28 and CP47 were co-purified with the stress-induced Small CAB-like Protein (Scp) D that is associated most prominent with the monomeric form of PSII [88]. The authors propose that Psb28 might stabilize the binding between CP47 and ScpD in monomeric PSII complexes under stress conditions.

In summary, Psb28 is substoichiometrically associated with PSII at the stromal side, namely RC47 core complexes and the CP47 protein. It is likely to be involved in CP47 biosynthesis and therefore PSII biogenesis.

3.3. Psb29/Thf1

The Psb29 protein is alternatively designated as Thf1 for Thylakoid Formation Protein 1 or ALC1 (Altered Coronatine Response) in *Solanum lycopersicum* and *Nicotiana benthamiana*[89]. While attempting to identify all PSII proteins, Pakrasi and his colleagues have employed proteomic technologies and found 31 proteins in a highly active PSII preparation from a genetically modified strain HT-3 of *Synechocystis*[62]. One of the five newly identified proteins, which is sub-stoichiometrically associated with PSII, was designated as Psb29 protein (Sll1414 in Cyanobase database) [62,90]. Other proteomic studies found the protein to be located in multiple compartments of the chloroplast [91–94]. It has been detected in proteomes of chloroplast envelopes [91], thylakoid membranes [93] and stroma [94]. *In vivo* localization identified Psb29-GFP (or Thf1-GFP) fusion protein in chloroplasts and stromules [95,96]. *In vitro* import and fractionation assays further suggested it to be mainly present in the membrane fraction, only a minor part was detected in the soluble fraction, likely in stroma [95,96]. The fraction associated with membranes was found to be protease-sensitive [95] and could be removed from PSII preparations by treatment with high salt [90], indicating that it is an extrinsic membrane protein associated with thylakoid membranes at the stromal side. Alternatively, this protein is predicted to contain two transmembrane helixes and was shown to be localized in the outer envelope of plastids [96]. The multiple locations of the protein in plastids may reflect its versatile roles in this organelle, which is indicated by several examples reported recently.

The Psb29 protein is highly conserved between cyanobacteria and flowering plants [90,95]. A homolog has even been found in an algal virus [95]. In *Synechocystis* the protein contains 240 amino acids with a molecular weight of 27.1 kDa. The *Arabidopsis* Psb29 protein is encoded by a nuclear gene, At2g20890; its precursor protein is 300 amino acids in length and its transit peptide is predicted to be 67 amino acids at the N-terminus. The mature form of the protein is 26.8 kDa. Expression of the protein is light regulated in a way similar to that of the light harvesting protein [95].

A Psb29 mutant has been generated in *Synechocystis*[90]. Deletion of this protein caused slower growth and lower PSII activity, especially under high light conditions. Moreover, uncoupling of antenna complexes from the PSII-core complex is more pronounced under high light in the mutant than in wild type. Electron microscopy revealed that the ultrastructure of thylakoids in wild type and the knockout mutant is similar [90].

The Psb29 T-DNA insertion mutant in *Arabidopsis* has been studied by a number of groups [90,95–97]. The mutant plants are stunted with variegated leaves and slower growth rates. Pakrasi and his colleagues focused on investigating PSII parameters of the mutant. They demonstrated that the PSII efficiency and coupled proximal antenna complexes in PSII of the *Arabidopsis* mutant were more susceptive to high light, similar to its cyanobacterial counterpart [90]. They also showed that the variegated phenotype could be rescued when the plants are grown under low light [90]. Another group led by Korth [95] has shown changes in ultrastructure of chloroplasts of the mutant. They showed that the tissues from the green sectors of mutant leaves had reduced amounts of thylakoid stacking, while most plastids in yellow/white sectors of the mutant lacked thylakoid membranes and starch granules. Moreover, these plastids accumulated membrane vesicles of various sizes. Antisense transgenic plants were generated to confirm that the structural defects were caused by low amounts of the Psb29/Thf1 protein in the transgenic plant, therefore indicating a role of Psb29/Thf1 in thylakoid biogenesis and leaf development, especially at early stages [95]. They designated the protein as the Thylakoid Formation Protein 1 or Thf1. Nevertheless, the authors did not exclude the possibility of indirect involvement of the protein in vesicle-mediated formation of thylakoid membranes [95].

In vitro and *in vivo* studies on the sugar signal pathway in *Arabidopsis* roots showed that the outer envelope localized Psb29/Thf1 interacted with GPA1, a plasma membrane-delimited G-protein α-subunit [96]. The *thf1* mutant is hypersensitive to high concentration of D-glucose. In addition, Psb29/Thf1 is degraded by treating roots with D-glucose. Therefore it is considered as a component in the G-protein-coupled sugar-signaling pathway in *Arabidopsis* roots [96].

In a recent report, D1 protein was shown to be more stable in the *thf1* mutant than in wild type because the levels of FtsH proteases were decreased in this mutant. FtsH complexes are known to be involved in repairing the photodamaged D1 protein of PSII [98–101]. Expression of a constitutively activated (GTPase-deficient) form of GPA1 in the *thf1* mutant background partially rescued the *thf1* mutant phenotype including FtsH amounts, variegated leaves, structural defects

and PSII activities [97]. Huang and his colleagues conclude that Thf1 functions as a regulator for FtsH expression via G protein-dependent and/or -independent pathways. In the dependent pathway, the chloroplast envelope-localized Thf1 might interact with the heterotrimeric G-protein, which in turn could regulate the expression of FtsH proteases. In the G-protein independent pathway, the stromal and thylakoid-localized Thf1 could affect the stability of FtsH protease, which then would lead to normal chloroplast development [97]. Another function proposed for Thf1 achieved via heterotrimeric G-protein is its involvement in phytochrome A-mediated cell death in *Arabidopsis*[102].

Besides the three functional pathways mentioned above, it is suggested that Thf1 may be also involved in other signal transduction pathways. High-throughput, semi-automated yeast two-hybrid interaction screening demonstrated that the Psb29/Thf1 interacted with two *Arabidopsis* Calcium-dependent protein kinases (AtCDPKs) AtCPK4 and AtCPK11 [103]. The two kinases are shown to have roles in the abscisic acid signal transduction pathway [104]. The physiological significance of these interactions is not clear at the present. Another recent striking finding is that Psb29/Thf1 takes a part in bacterial speck disease development, however, its role in the process is not clear [89,105].

To summarize the function of the Psb29/Thf1, we propose that the thylakoid-localized portion of Psb29/Thf1 may play a regulatory role in PSII, which is more important under high light, given that the Psb29/Thf1 protein is associated with PS II at sub-stoichiometric levels and is not involved in electron transfer in the PSII core. It seems that the protein fulfills its function through regulating the stability/activity of FtsH proteases to affect the photodamage and photorepair cycle of the D1 protein and therefore influences PSII biogenesis.

3.4. Psb30

The searching for Psb30 started in 2005, when the PS II crystal structure of *T. elongates* resolved at 3.0 Å had been reported [4]. This remarkable PSII structure model assigned 11 transmembrane helices (TMH) for PsbE, PsbF, PsbH, PsbI, PsbJ, PsbK, PsbL, PsbM, PsbTc and PsbZ (2 TMHs), leaving three undefined, named X1, *X*2 and X3. Prompted by the new findings, Kashino and his coworkers speculated that the *X*2 would be PsbY and X3 would be PsbX, and the X1 might be an unidentified PSII component [106]. After isolating PSII core complexes with high oxygen-evolving activities from this organism, a new component was identified. The protein is encoded by *ycf12* (hypothetical chloroplast open reading frame) and is designated as Psb30 [106]. In *Synechocystis* 6803, the His-tagged Psb30 (Sll0047) was found to interact with PSII core components [107], though the protein itself was poorly stained by Coomassie Blue and was hard to detect by antibodies directed against poly-histidine.

The Psb30 protein is conserved from cyanobacteria to land plants except for some marine cyanobacteria and angiosperms, for example, *Amborella trichopoda*[108], *Arabidopsis* and rice [109]. It is plastid-encoded in eukaryotes. Psb30 of various species contains 32–46 amino acids in length and has a molecular weight ranging from 3.3 to 5.0 kDa. The *Synechocystis* Psb30 is predicted to be much bigger, consisting of 93 amino acids. The small protein has a single TMH and is therefore highly hydrophobic.

Knockout mutants of this protein have been generated in *Synechocystis* [107] and *T. elongatus* [57,109]. The Psb30-deletion mutant of *Synechocystis* can grow photoautotrophically and is not distinguishable from wild type under normal growth conditions. However, mutant PSII complexes display lower oxygen-evolving activities under high light intensities, indicating that the Psb30 protein has a role in optimizing PSII activity under strong irradiance [107]. The Psb30-deletion mutants of the thermophilic cyanobacterium *T. elongatus* were generated by two laboratories [57,109]. The oxygen-evolving activities of the mutant thylakoid membranes and PSII dimers isolated by Takatsaka and his colleagues were lower than those of wild type [57], due to the lower stability and amounts of PSII dimer complexes in mutant thylakoids. However, loss of Psb30 in PSII dimers did not result in loss of other PSII components or structural change of the PSII complexes [57].

The oxygen-evolving rates of the mutant PSII complexes prepared by Sugiura and her colleagues are comparable to that of wild type under saturating continuous illumination [109]. However, high light treatment of cells results in difference in oxygen-evolving activities between wild type and the mutant, indicating that the mutant is more susceptible to photodamage [109]. The reason for this higher susceptibility of the mutant to high light is likely because the Cyt b_{559} in the isolated thylakoids from the mutant is in its low potential form. Cyt b_{559} is known as a component in the photo-induced side-pathway of electron transport in PSII. It exists at least in two forms, a high potential form that is dominant in intact PSII complexes and a low potential form that results in oxidation of heme under ambient redox conditions [110,111]. It is noteworthy that the MALDI-TOF mass spectra of the isolated PSII complexes from this Psb30-deletion mutant do not display the PsbY protein peak [109], suggesting that some phenotype of the Psb30 mutant might be the looser association of the PsbY with PSII.

The location of the Psb30 protein in PSII super-complexes was confirmed by comparing the crystal structures of the mutant and wild type PSII dimers [57], which is consistent with the position of the protein determined in the structure at 2.9 Å resolution [2]. The protein is adjacent to PsbJ, PsbK and PsbZ in the PSII dimer.

Studies using *psbK*[15] and *psbZ*[58] mutants in the same organism also suggest that the Psb30 protein associates with PSII through interacting with these two proteins because the Psb30 protein is completely lost in PSII complexes isolated from either mutant. These findings are in line with the latest publication of the PSII structure [112,6]. No mutants are available in organisms other than cyanobacteria and no functional studies on this protein in other organisms either.

In conclusion, Psb30 is likely to have both structural and functional roles in PSII; a component to protect PSII, presumably by preventing Cyt b_{559} from converting to low potential form under high light and to stabilize PSII dimer. However, its contribution to PSII stability might be indirectly, given that it is located at the opposite position of the monomer–monomer interface.

3.5. Psb31

The Psb31 protein was initially found to be part of highly active PSII particles from the diatom *Chaetoceros gracilis* by Enami and his colleagues in 2007 [113]. The isolated PSII particles contain five extrinsic proteins, among which four are red alga-like, PsbO, PsbQ′, PsbU and PsbV and the fifth is a novel protein, designated Psb31 [114]. When the PSII complexes are further purified by removing most fucoxanthin chlorophyll *a*/*c*-binding proteins, Psb31 is still present in the PSII preparations, suggesting that it is a PSII component in this organism [115].

Psb31 from this marine diatom has 124 amino acid residues and its molecular weight is 13.3 kDa [114]. The protein is nuclear encoded. The cDNA of this gene has been cloned and the deduced amino acid sequence displays three characteristic signal peptides that are 55 amino acids and target the protein through chloroplast endoplasmic reticulum, chloroplast envelope and thylakoid membrane to the lumen [114]. The Psb31 protein is associated with PSII through electrostatic interaction in the lumenal side of thylakoid membrane. The nearest neighbors in PSII core of the protein as determined by using cross-linker EDC are proteins PsbH or/and Cyt b_{559}. Its secondary structure is predicted to form a three-helical bundle structure [114].

Homologous genes are found in red algae and chromophytic algae, but not in other organisms. Phylogenetic and biochemical analyses suggest that only the Psb31 protein in diatom and maybe in pelagophyceae and brown algae work with other four PSII extrinsic proteins, since the Psb31 protein is not found to be associated with PSII complexes that retain high oxygen evolving activity isolated from a red alga, *Cyanidium caldarium* [116,117].

There have been no mutants generated so far for loss-of-function studies. However, using biochemical approaches, the role of the Psb31 protein has been investigated [118]. The Psb31 protein and the other four extrinsic proteins, PsbO, PsbQ′, PsbU and PsbV have been purified and reconstituted with the PSII intrinsic complex. The Psb31 protein can directly bind to PSII intrinsic complexes in a manner similar to that of PsbO and PsbQ′. In addition, these studies suggest that the Psb31 protein can partially replace the function of PsbO that is important for maintaining the stability and activity of Mn_4Ca cluster in the oxygen-evolving complex of PSII.

3.6. Psb32/TLP18.3

Psb32 was initially detected in a highly active PSII preparation of the cyanobacterium *Synechocystis* 6803 (Sll1390) [62] and in the proteome of chloroplast thylakoids (At1g54780) [72,73]. It is conserved in all oxygenic photosynthetic organisms that contain thylakoid membranes [119] and belongs to UPF0603 family with a domain of unknown function (DUF477). It contains a transmembrane span at the C-terminus; the majority of the protein is localized in the thylakoid lumen. The precursor protein possesses a transit-peptide of 46 and 84 amino acids and the theoretical molecular masses of the mature proteins are 22.4 and 22.2 kDa in *Synechocystis* and *Arabidopsis*, respectively. Nevertheless, its molecular mass measured by MS is 18.3 kDa in *Arabidopsis*, hence it was named TLP18.3 indicating its location (thylakoid lumenal protein) and size [72]. The protein is predicted to translocate across thylakoid membranes via Sec pathway [73].

The function of Psb32 has been studied in *Synechocystis* 6803 [119]. The Psb32 deletion mutant displays more severe photoinhibition and slower recovery rates than wild type. Psb32 was also shown to protect cells from oxidative stress [119].

In Arabidopsis Psb32/TLP18.3 was detected in the proteome of thylakoid-associated polysome nascent chain complexes [120]. The protein is near evenly distributed in the stroma-exposed thylakoids and grana stacks. Two T-DNA insertion lines of Psb32 do not display a viable phenotype under normal growth conditions or high light intensities, but they are smaller than the wild type when grown under fluctuating light. Nevertheless, the mutant lines seem to be more susceptible to high light treatment caused by insufficient PSII repair compared to wild type. Further studies demonstrated that the protein was involved in turnover of D1 protein [120]. Lack of Psb32 also causes up-regulation of the oxygen-evolving complex proteins PsbP and PsbQ, and increased amounts of PSII monomers. Aro and coworkers concluded that Psb32/TLP18.3, serving as an auxiliary protein of PSII, plays a functional role in the PSII repair cycle in stroma-exposed thylakoids and in the assembly of PSII monomers to dimers in thylakoid stacks [120,121].

3.7. Stress-induced low molecular weight proteins associated with Photosystem II

In the cyanobacterium *Synechocystis* 6803 a group of low molecular weight proteins has been found to associate with Photosystem II when the cells were exposed to various stresses (Table 1). They were originally named Hlips, for “high-light inducible proteins” [122], however, they are induced not only under high light, but also during other stresses including low temperature, and N- and S-starvation [123,124]. Therefore they are also referred to as SCPs (for “small CAB-like proteins”) in *Synechocystis* 6803 [125], based on their homology to the chlorophyll *a*/*b* binding (CAB) domain of the first and third helix of the higher plant light harvesting complex and may be similar to the evolutionary ancestor of the CAB proteins [126]. They belong to an extended family of light-harvesting-like (Lil) proteins, that instead of harvesting light energy is thought to be involved in light protection [138]. The genomes of all photoautotrophic organisms investigated so far contain genes coding for these one-helix Lil proteins, however, the large family of Lil proteins in eukaryotes additionally consists of proteins predicted to have two, three and/or four membrane spanning helices (reviewed in [127]. In the small genome of the cyanobacterium *Prochlorococcus marinus* MED4 as many as 23 *scp* or *hli* genes were identified [128] and recently these genes have been detected in genomes of *Prochlorococcus* cyanophages [129,130], where they are believed to maintain the photosynthetic activity of the host during an infection [129]. Although the function of the small CAB-like proteins is not fully understood, these findings indicate their importance.

In the cyanobacterium *Synechocystis* 6803, five SCPs were identified [125]; four of them (ScpB-E) encode proteins of around 6 kDa, while the fifth one (ScpA) is the C-terminal extension of the ferrochelatase (HemH). A mutant with ScpB-E genes inactivated was sensitive to high-intensity illumination and showed alteration in pigmentation and in the ability to perform non-photochemical dissipation of absorbed light energy [131]. It was suggested that SCPs play a role in non-photochemical quenching (NPQ) [131], but absence of *scp* genes does not impair fluorescence characteristics [132,145]. Instead a soluble orange carotenoid protein (OCP) has been recognized to be the main player in cyanobacterial NPQ (reviewed by [133]).

The presence of the CAB motif in SCPs suggested that they may be able to bind Chl molecules, which also could be shown *in vitro*[134]. Their importance to stabilize chlorophyll-binding proteins is well documented [132,135–137], they might be important for the tetrapyrrole metabolic pathway [132,135,138,139,145] or act as pigment-carriers [91,125,136]. Using affinity chromatography, 2D PAGE or electron microscopy, ScpB-D have been found to be located close to Photosystem II (PSII), they seem to bind to CP47 and/or Psb28 [140,141,146]. The location of ScpE still is not entirely clarified [141,146]. Interestingly, ScpC and ScpD seem to be functionally complementary [132]. These two genes are most similar (87.1% identity [123]), indicating a rather recent gene duplication [128] or a reasonably strict primary structure requirement. ScpD has been shown to bind to CP47 [91], in the vicinity of PsbH [140]. Both CP47 and PsbH are forming a complex at the early stage in PSII assembly [142,143].

In eukaryotes the location of these small Lil proteins within the thylakoid membrane is not clear. In higher plants the name “OHP” (one-helix protein) is used for the nuclear-encoded small CAB-like proteins. Two distinct classes within this group evolved: OHP1/Lil2 and OHP2/Lil6 [127]. In *Arabidopsis* OHP2 has been found to associate with Photosystem I [144], the location of OHP1 is not known.

4. Are the low molecular mass proteins a specific subgroup of the PSII proteins?

To address this question co-expression analysis of genes encoding PSII proteins was performed using publicly available gene expression data from 4006 microarrays, obtained from the Nottingham *Arabidopsis* Stock Center's microarray database. Due to unspecificity of the array probes for the PsbD and PsbF protein genes they could not be included in the analysis, In co-expression analysis one searches for genes which show a similar expression profile across numerous different microarray experiments, representing different environmental and stress conditions as well as developmental stages and tissue types. This analysis method was applied to genes encoding the PSII proteins of *Arabidopsis thaliana*, to characterize differences and similarities in transcriptional

Table 1
Features of novel proteins of Photosystem II.

Protein	Other names	Gene	Length (amino acid)	Size (kDa)	Property	Location in PSII	Mutant	Function	Organism
Psb27	11 kDa protein, Psb27-H1 Psb27-H2 Lpa19 (PsbZ)	*slr1645* At1g03600 At1g05385	110[a] 107[b] 135[b]	12.3 kDa[a] 11.8 kDa[b] 15.1 kDa[b]	Extrinsic	In PSII monomer Lumenal side	*Synechocystis* 6803 *Arabidopsis*	D1 C-terminal processing Mn_4Ca assembly PSII assembly PSII repair	Conserved from cyanobacteria to flowering plants.
Psb28	Ycf79 Psb13 (PsbW)	*sll1398* At4g28660	112[a] 134[b]	12.6 kDa[a] 15.1 kDa[b]	Extrinsic	In PSII monomer (RC47) Stromal side	*Synechocystis* 6803	CP47 biogenesis PSII assembly	Conserved from cyanobacteria to flowering plants.
Psb29	Thf1 ALC1	*sll1414* At2g20890	240[a] 233[b]	27.1 kDa[a] 26.8 kDa[b]	Intrinsic[c] and extrinsic[d]	Stromal side	*Synechocystis* 6803 *Arabidopsis*	Regulation in photodamage and photorepair cycle	Conserved from cyanobacteria to flowering plants.
Psb30	Ycf12	*sll0047*	32–46	3.3–5.0 kDa	One TMH	Adjacent to PsbK, PsbJ and PsbZ	*T. elongates*, *Synechocystis* 6803	Stabilize PSII dimer; Prevent Cytb559 from converting to low potential form under high light	Conserved from cyanobacteria to land plants. Not in angiosperms and certain marine cyanobacteria.
Psb31			113–203	12.3–21.8 kDa	Extrinsic	In OEC at lumenal side	No mutants are available	Supporting oxygen-evolving	Only red algae and chromophytic algae
Psb32	TLP18.3	Sll1390 At1g54780	203[a] 201[b]	22.4 kDa[a] 32.2 kDa[b]	Intrinsic	Major portion is on lumenal side	*Synechocystis* 6803 *Arabidopsis*	Protection oxidative stress Repair PSII Assembly PSII dimer	Conserved from cyanobacteria to flowering plants.
ScpD	HliB	*ssr2595*	70	6 kDa	Intrinsic	Close to CP47	*Synechocystis* 6803	Stress-induced,	Relatives in all organisms performing oxygenic photosynthesis and in cyanophages
ScpC	HliA	*ssl2542*	70	6 kDa	Intrinsic	n.d.		Chl stabilizator,	
ScpB	HliC	*ssl1633*	47	4 kDa	Intrinsic	n.d.		pigment carrier?	

TMH: transmembrane helix.
Protein names in brackets are renamed according to a modified nomenclature. PsbW and PsbZ are two proteins distinct from Psb27 and Psb28, respectively [9,62].
[a] *Synechocystis* 6803.
[b] Mature form in *Arabidopsis thaliana*.
[c] See [96].
[d] See [90].

expression among the PSII proteins themselves. In order to study the expression pattern and not the expression level for the selected genes the data was scaled gene-wise to unit variance. The scaled dataset was thereafter analyzed by principal component analysis (PCA) using SIMCA-P 12.01 (Umetrics AB, Umeå, Sweden). For clarity we have used protein nomenclature in the figure and analysis although it is gene expression which is discussed. The PCA score plot (Fig. 2) reveals two main gene clusters separated on the second component: one cluster consisting of genes solely from the chloroplast genome (blue) and another cluster consisting of genes solely from the nuclear genome (red). This suggests that the chloroplast genes and the nuclear genes are responding differently on different environmental and stress conditions as well as developmental stages and tissue types. This is in agreement with an earlier report [49] where it was shown that several nuclear-encoded thylakoid proteins such as Lhcb and PsbW were synthesized only under low light intensities, whereas high light intensities favored the synthesis and assembly of chloroplast-encoded PSII proteins.

Both the chloroplast and the nuclear PSII gene clusters contain both larger PSII reaction center protein genes as well as low molecular mass protein genes. Thus it seems clear that the low molecular mass protein genes are not a specific subgroup of genes with a distinct common expression pattern. It is interesting to note that the Lil protein genes, especially Lil5, are distinctly separated from the major nuclear encoded protein genes in their transcriptional expression pattern. Also the new components Psb27 and 28 are separated from the major expression pattern of the nuclear PSII genes, suggestion that they have a distinct regulation compared to other nuclear PSII genes. Looking at the chloroplastic genes four proteins have a somewhat different expression pattern, PsbH, PsbM, PsbTc and PsbN. In the latter case it has been questioned if PsbN is a PSII component or not [62] which would explain that it is an outlier in the co-expression analysis. From this analysis it seems clear that the low molecular proteins of PSII are not forming a separate subgroup with respect to their transcriptional regulation, but are normal integrated components of PSII. It seems to be more important if the gene is chloroplast or nuclear encoded indicating that the key factors that coordinate PSII as a whole functional complex are posttranscriptional.

5. Concluding remarks

Of the six newly identified proteins discussed above, only two of them, Psb30 and Psb31, are stable structural components of PSII supercomplexes. Psb30 is conserved from cyanobacteria through land plants, but is absent in angiosperms. It is not clear if there is a counterpart of Psb30 in angiosperms. A PSII 3D-structure map at high resolution from such plants will answer this question. The Psb31 protein is only present in organisms of red alga linkage. It will be interesting to find out why only such a narrow spectrum of organisms require Psb31 for their maximal PSII activities.

The other four proteins are either transiently associated with PSII at the lumenal side of the thylakoid membrane (Psb27, Psb32), or substoichiometrically associated with PSII at the stromal/cytoplasmic side (Psb28, Psb29). They may not be part of the final functional PSII complexes; instead, they appear to play a role in the constant assembly and disassembly of PSII complexes. Purification and characterization of PSII complexes varying in assembly stages will help to elucidate their precise roles, as has already been done for the Psb27 protein.

With respect to nomenclature, some of these proteins may eventually be renamed depending on their exact functions as revealed in the future. It is likely that at least some of them will be classified as regulatory factors in PSII, making their Psb designations inappropriate.

An intriguing observation concerns the different roles of low molecular mass proteins in prokaryotes and eukaryotes: deletion/removal of some low molecular proteins has more detrimental effects in eukaryotic organisms compared to prokaryotic. It is possible that these proteins are located at a different site in eukaryotes – hopefully PSII structures from eukaryotic organisms will reveal new insight – or due to their evolutionary longer existence and simpler structure prokaryotes have a better network for compensation of missing components.

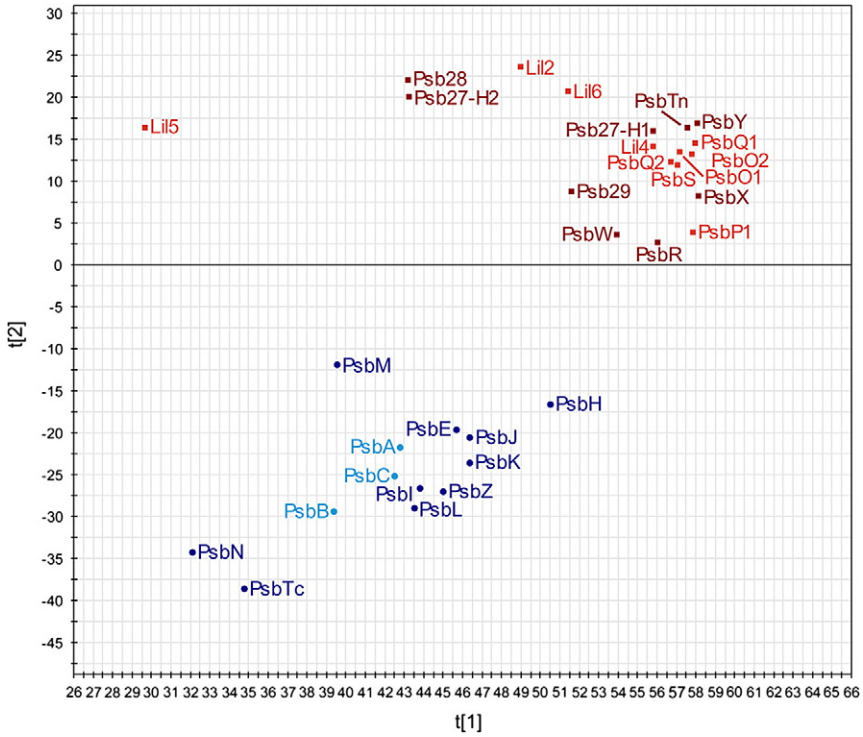

Fig. 2. Principal component analysis of scaled gene expression data from 4006 arrays for PSII and Lil genes, with the exception of PsbD and PsbF for which data was not available. Chloroplast genome genes are denoted in blue and genes from the nuclear genome in red. Low molecular mass proteins are colored dark, *i.e.* dark blue and dark red, while all other components are colored light, *i.e.* light blue and light red. For simplicity protein names rather than gene names are used.

Acknowledgment

We thank Dr. Steven Theg for valuable discussion on this manuscript. We are also grateful to Dr. Xiao-Ping Zhang for advising on the preparation of Fig. 1 (PDB code: 3ARC). The work was supported by the Swedish Energy Agency and Umeå University (to C.F.), the Swedish Research Council (to W.P.S.) (VR, 621-2008-3207). C.F. would like to thank the Royal Swedish Academy for granting her research position.

References

[1] K.N. Ferreira, T.M. Iverson, K. Maghlaoui, J. Barber, S. Iwata, Architecture of the photosynthetic oxygen-evolving center, Science 303 (2004) 1831–1838.
[2] A. Guskov, J. Kern, A. Gabdulkhakov, M. Broser, A. Zouni, W. Saenger, Cyanobacterial photosystem II at 2.9-A resolution and the role of quinones, lipids, channels and chloride, Nat. Struct. Mol. Biol. 16 (2009) 334–342.
[3] N. Kamiya, J.R. Shen, Crystal structure of oxygen-evolving photosystem II from *Thermosynechococcus vulcanus* at 3.7-A resolution, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 98–103.
[4] B. Loll, J. Kern, W. Saenger, A. Zouni, J. Biesiadka, Towards complete cofactor arrangement in the 3.0 A resolution structure of photosystem II, Nature 438 (2005) 1040–1044.
[5] A. Zouni, H.T. Witt, J. Kern, P. Fromme, N. Krauss, W. Saenger, P. Orth, Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 A resolution, Nature 409 (2001) 739–743.
[6] Y. Umena, K. Kawakami, J.R. Shen, N. Kamiya, Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 A, Nature 473 (2011) 55–60.
[7] J.P. Dekker, E.J. Boekema, Supramolecular organization of thylakoid membrane proteins in green plants, Biochim. Biophys. Acta 1706 (2005) 12–39.
[8] F.A. Wollman, L. Minai, R. Nechushtai, The biogenesis and assembly of photosynthetic proteins in thylakoid membranes1, Biochim. Biophys. Acta 1411 (1999) 21–85.
[9] L.X. Shi, W.P. Schroder, The low molecular mass subunits of the photosynthetic supracomplex, photosystem II, Biochim. Biophys. Acta 1608 (2004) 75–96.
[10] B. Granvogl, M. Zoryan, M. Ploscher, L.A. Eichacker, Localization of 13 one-helix integral membrane proteins in photosystem II subcomplexes, Anal. Biochem. 383 (2008) 279–288.
[11] M. Ploscher, B. Granvogl, M. Zoryan, V. Reisinger, L.A. Eichacker, Mass spectrometric characterization of membrane integral low molecular weight proteins from photosystem II in barley etioplasts, Proteomics 9 (2009) 625–635.
[12] P. Kunstner, A. Guardiola, Y. Takahashi, J.D. Rochaix, A mutant strain of *Chlamydomonas reinhardtii* lacking the chloroplast photosystem II psbI gene grows photoautotrophically, J. Biol. Chem. 270 (1995) 9651–9654.
[13] M. Ikeuchi, V.K. Shukla, H.B. Pakrasi, Y. Inoue, Directed inactivation of the psbI gene does not affect photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803, Mol. Gen. Genet. 249 (1995) 622–628.
[14] S. Schwenkert, P. Umate, C. Dal Bosco, S. Volz, L. Mlcochova, M. Zoryan, L.A. Eichacker, I. Ohad, R.G. Herrmann, J. Meurer, PsbI affects the stability, function, and phosphorylation patterns of photosystem II assemblies in tobacco, J. Biol. Chem. 281 (2006) 34227–34238.
[15] M. Iwai, T. Suzuki, A. Kamiyama, I. Sakurai, N. Dohmae, Y. Inoue, M. Ikeuchi, The PsbK subunit is required for the stable assembly and stability of other small subunits in the PSII complex in the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1, Plant Cell Physiol. 51 (2010) 554–560.
[16] M. Ikeuchi, B. Eggers, G.Z. Shen, A. Webber, J.J. Yu, A. Hirano, Y. Inoue, W. Vermaas, Cloning of the *psbK* gene from *Synechocystis* sp. PCC 6803 and characterization of photosystem II in mutants lacking PSII-K, J. Biol. Chem. 266 (1991) 11111–11115.
[17] Y. Takahashi, H. Matsumoto, M. Goldschmidt-Clermont, J.D. Rochaix, Directed disruption of the *Chlamydomonas* chloroplast *psbK* gene destabilizes the photosystem II reaction center complex, Plant Mol. Biol. 24 (1994) 779–788.
[18] S. Naithani, P.K. Trivedi, R. Tuli, P.V. Sane, The psbE-F-L-J operon from chloroplast genome of *Populus deltoides*: cloning, nucleotide sequence and transcript analysis, J. Genet. 76 (1997) 61–72.
[19] F. Morais, J. Barber, P.J. Nixon, The chloroplast-encoded alpha subunit of cytochrome b-559 is required for assembly of the photosystem two complex in both the light and the dark in *Chlamydomonas reinhardtii*, J. Biol. Chem. 273 (1998) 29315–29320.
[20] H.B. Pakrasi, P. De Ciechi, J. Whitmarsh, Site directed mutagenesis of the heme axial ligands of cytochrome b559 affects the stability of the photosystem II complex, EMBO J. 10 (1991) 1619–1627.
[21] H.B. Pakrasi, K.J. Nyhus, H. Granok, Targeted deletion mutagenesis of the beta subunit of cytochrome b559 protein destabilizes the reaction center of photosystem II, Z. Naturforsch. C 45 (1990) 423–429.
[22] M. Swiatek, R.E. Regel, J. Meurer, G. Wanner, H.B. Pakrasi, I. Ohad, R.G. Herrmann, Effects of selective inactivation of individual genes for low-molecular-mass subunits on the assembly of photosystem II, as revealed by chloroplast transformation: the psbEFLJ operon in *Nicotiana tabacum*, Mol. Genet. Genomics 268 (2003) 699–710.
[23] M. Suorsa, R.E. Regel, V. Paakkarinen, N. Battchikova, R.G. Herrmann, E.M. Aro, Protein assembly of photosystem II and accumulation of subcomplexes in the absence of low molecular mass subunits PsbL and PsbJ, Eur. J. Biochem. 271 (2004) 96–107.
[24] F. Muh, T. Renger, A. Zouni, Crystal structure of cyanobacterial photosystem II at 3.0 A resolution: a closer look at the antenna system and the small membrane-intrinsic subunits, Plant Physiol. Biochem. 46 (2008) 238–264.
[25] R.E. Regel, N.B. Ivleva, H. Zer, J. Meurer, S.V. Shestakov, R.G. Herrmann, H.B. Pakrasi, I. Ohad, Deregulation of electron flow within photosystem II in the absence of the PsbJ protein, J. Biol. Chem. 276 (2001) 41473–41478.
[26] I. Ohad, C. Dal Bosco, R.G. Herrmann, J. Meurer, Photosystem II proteins PsbL and PsbJ regulate electron flow to the plastoquinone pool, Biochemistry 43 (2004) 2297–2308.
[27] L.K. Lind, V.K. Shukla, K.J. Nyhus, H.B. Pakrasi, Genetic and immunological analyses of the cyanobacterium *Synechocystis* sp. PCC 6803 show that the protein encoded by the psbJ gene regulates the number of photosystem II centers in thylakoid membranes, J. Biol. Chem. 268 (1993) 1575–1579.
[28] M. Hager, M. Hermann, K. Biehler, A. Krieger-Liszkay, R. Bock, Lack of the small plastid-encoded PsbJ polypeptide results in a defective water-splitting apparatus of photosystem II, reduced photosystem I levels, and hypersensitivity to light, J. Biol. Chem. 277 (2002) 14031–14039.
[29] P.R. Anbudurai, H.B. Pakrasi, Mutational analysis of the PsbL protein of photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803, Z. Naturforsch. C 48 (1993) 267–274.
[30] H. Luo, J.J. Eaton-Rye, Directed mutagenesis of the transmembrane domain of the PsbL subunit of photosystem II in *Synechocystis* sp. PCC 6803, Photosynth. Res. 98 (2008) 337–347.
[31] T. Nagatsuka, S. Fukuhara, K. Akabari, Y. Toyoshima, Disintegration and recombination of photosystem II reaction center core complex. II. Possible involvement of low-molecular-mass proteins in the functioning of Q_A in the PSII reaction center, Biochim. Biophys. Acta 1057 (1991) 223–231.
[32] K. Kitamura, S. Ozawa, T. Shiina, Y. Toyoshima, L protein, encoded by psbL, restores normal functioning of the primary quinone acceptor, QA, in isolated D1/D2/CP47/Cytb-559/I photosystem II reaction center core complex, FEBS Lett. 354 (1994) 113–116.
[33] H. Hoshida, R. Sugiyama, Y. Nakano, T. Shiina, Y. Toyoshima, Electron paramagnetic resonance and mutational analyses revealed the involvement of photosystem II-L subunit in the oxidation step of Tyr-Z by P680+ to form the Tyr-Z+ P680Pheo- state in photosystem II, Biochemistry 36 (1997) 12053–12061.
[34] U. Ljungberg, H.E. Akerlund, B. Andersson, Isolation and characterization of the 10-kDa and 22-kDa polypeptides of higher plant photosystem 2, Eur. J. Biochem. 158 (1986) 477–482.
[35] M. Suorsa, S. Sirpio, Y. Allahverdiyeva, V. Paakkarinen, F. Mamedov, S. Styring, E.M. Aro, PsbR, a missing link in the assembly of the oxygen-evolving complex of plant photosystem II, J. Biol. Chem. 281 (2006) 145–150.
[36] P. Umate, S. Schwenkert, I. Karbat, C. Dal Bosco, L. Mlcochova, S. Volz, H. Zer, R.G. Herrmann, I. Ohad, J. Meurer, Deletion of PsbM in tobacco alters the QB site properties and the electron flow within photosystem II, J. Biol. Chem. 282 (2007) 9758–9767.
[37] F.K. Bentley, H. Luo, P. Dilbeck, R.L. Burnap, J.J. Eaton-Rye, Effects of inactivating psbM and psbT on photodamage and assembly of photosystem II in *Synechocystis* sp. PCC 6803, Biochemistry 47 (2008) 11637–11646.
[38] K. Kawakami, Y. Umena, M. Iwai, Y. Kawabata, M. Ikeuchi, N. Kamiya, J.R. Shen, Roles of PsbI and PsbM in photosystem II dimer formation and stability studied by deletion mutagenesis and X-ray crystallography, Biochim. Biophys. Acta 1807 (2011) 319–325.
[39] N. Ohnishi, Y. Takahashi, PsbT polypeptide is required for efficient repair of photodamaged photosystem II reaction center, J. Biol. Chem. 276 (2001) 33798–33804.
[40] N. Ohnishi, Y. Kashino, K. Satoh, S. Ozawa, Y. Takahashi, Chloroplast-encoded polypeptide PsbT is involved in the repair of primary electron acceptor QA of photosystem II during photoinhibition in *Chlamydomonas reinhardtii*, J. Biol. Chem. 282 (2007) 7107–7115.
[41] N. Ohnishi, Y. Takahashi, Chloroplast-encoded PsbT is required for efficient biogenesis of photosystem II complex in the green alga *Chlamydomonas reinhardtii*, Photosynth. Res. 98 (2008) 315–322.
[42] P. Umate, C. Fellerer, S. Schwenkert, M. Zoryan, L.A. Eichacker, A. Sadanandam, I. Ohad, R.G. Herrmann, J. Meurer, Impact of PsbTc on forward and back electron flow, assembly, and phosphorylation patterns of photosystem II in tobacco, Plant Physiol. 148 (2008) 1342–1353.
[43] M. Iwai, H. Katoh, M. Katayama, M. Ikeuchi, PSII-Tc protein plays an important role in dimerization of photosystem II, Plant Cell Physiol. 45 (2004) 1809–1816.
[44] K.D. Irrgang, L.X. Shi, C. Funk, W.P. Schroder, A nuclear-encoded subunit of the photosystem II reaction center, J. Biol. Chem. 270 (1995) 17588–17593.
[45] L.X. Shi, W.P. Schröder, Compositional and topological studies of the PsbW protein in spinach thylakoid membrane, Photosynth. Res. 53 (1997) 45–53.
[46] L.X. Shi, Z.J. Lorkovic, R. Oelmuller, W.P. Schroder, The low molecular mass PsbW protein is involved in the stabilization of the dimeric photosystem II complex in *Arabidopsis thaliana*, J. Biol. Chem. 275 (2000) 37945–37950.
[47] E. Thidholm, V. Lindstrom, C. Tissier, C. Robinson, W.P. Schroder, C. Funk, Novel approach reveals localisation and assembly pathway of the PsbS and PsbW proteins into the photosystem II dimer, FEBS Lett. 513 (2002) 217–222.
[48] E.J. Boekema, J.F. van Breemen, H. van Roon, J.P. Dekker, Arrangement of photosystem II supercomplexes in crystalline macrodomains within the thylakoid membrane of green plant chloroplasts, J. Mol. Biol. 301 (2000) 1123–1133.
[49] A. Rokka, M. Suorsa, A. Saleem, N. Battchikova, E.M. Aro, Synthesis and assembly of thylakoid protein complexes: multiple assembly steps of photosystem II, Biochem. J. 388 (2005) 159–168.
[50] J.G. Garcia-Cerdan, L. Kovacs, T. Toth, S. Kereiche, E. Aseeva, E.J. Boekema, F. Mamedov, C. Funk, W.P. Schroder, The PsbW protein stabilizes the supramolecular organization of photosystem II in higher plants, Plant J. 65 (2011) 368–381.

[51] L.X. Shi, S.J. Kim, A. Marchant, C. Robinson, W.P. Schröder, Characterisation of the PsbX protein from Photosystem II and light regulation of its gene expression in higher plants, Plant Mol. Biol. 40 (1999) 737–744.
[52] C. Funk, Functional analysis of the PsbX protein by deletion of the corresponding gene in *Synechocystis* sp. PCC 6803, Plant Mol. Biol. 44 (2000) 815–827.
[53] H. Katoh, M. Ikeuchi, Targeted disruption of psbX and biochemical characterization of photosystem II complex in the thermophilic cyanobacterium *Synechococcus elongatus*, Plant Cell Physiol. 42 (2001) 179–188.
[54] J.G. Garcia-Cerdan, D. Sveshnikov, D. Dewez, S. Jansson, C. Funk, W.P. Schröder, Antisense inhibition of the PsbX protein affects PSII integrity in the higher plant *Arabidopsis thaliana*, Plant Cell Physiol. 50 (2009) 191–202.
[55] K. Kawakami, M. Iwai, M. Ikeuchi, N. Kamiya, J.R. Shen, Location of PsbY in oxygen-evolving photosystem II revealed by mutagenesis and X-ray crystallography, FEBS Lett. 581 (2007) 4983–4987.
[56] A.E. Gau, H.H. Thole, A. Sokolenko, L. Altschmied, R.G. Hermann, E.K. Pistorius, PsbY, a novel manganese-binding, low-molecular-mass protein associated with photosystem II, Mol. Gen. Genet. 260 (1998) 56–68.
[57] K. Takasaka, M. Iwai, Y. Umena, K. Kawakami, Y. Ohmori, M. Ikeuchi, Y. Takahashi, N. Kamiya, J.R. Shen, Structural and functional studies on Ycf12 (Psb30) and PsbZ-deletion mutants from a thermophilic cyanobacterium, Biochim. Biophys. Acta 1797 (2010) 278–284.
[58] M. Iwai, T. Suzuki, N. Dohmae, Y. Inoue, M. Ikeuchi, Absence of the PsbZ subunit prevents association of PsbK and Ycf12 with the PSII complex in the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1, Plant Cell Physiol. 48 (2007) 1758–1763.
[59] C.L. Bishop, S. Ulas, E. Baena-Gonzalez, E.M. Aro, S. Purton, J.H. Nugent, P. Maenpaa, The PsbZ subunit of Photosystem II in *Synechocystis* sp. PCC 6803 modulates electron flow through the photosynthetic electron transfer chain, Photosynth. Res. 93 (2007) 139–147.
[60] S. Ruf, K. Biehler, R. Bock, A small chloroplast-encoded protein as a novel architectural component of the light-harvesting antenna, J. Cell Biol. 149 (2000) 369–378.
[61] M. Kobayashi, K. Okada, M. Ikeuchi, A suppressor mutation in the alpha-phycocyanin gene in the light/glucose-sensitive phenotype of the psbK-disruptant of the cyanobacterium *Synechocystis* sp. PCC 6803, Plant Cell Physiol. 46 (2005) 1561–1567.
[62] Y. Kashino, W.M. Lauber, J.A. Carroll, Q. Wang, J. Whitmarsh, K. Satoh, H.B. Pakrasi, Proteomic analysis of a highly active photosystem II preparation from the cyanobacterium *Synechocystis* sp. PCC 6803 reveals the presence of novel polypeptides, Biochemistry 41 (2002) 8004–8012.
[63] L. Wei, J. Guo, M. Ouyang, X. Sun, J. Ma, W. Chi, C. Lu, L. Zhang, LPA19, a Psb27 homolog in *Arabidopsis thaliana*, facilitates D1 protein precursor processing during PSII biogenesis, J. Biol. Chem. 285 (2010) 21391–21398.
[64] M. Ikeuchi, Y. Inoue, W. Vermaas (Eds.), Characterization of Photosystem II Subunits from the Cyanobacterium *Synechocystis* sp. PCC 6803, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1995.
[65] Y. Kashino, H. Koike, M. Yoshio, H. Egashira, M. Ikeuchi, H.B. Pakrasi, K. Satoh, Low-molecular-mass polypeptide components of a photosystem II preparation from the thermophilic cyanobacterium *Thermosynechococcus vulcanus*, Plant Cell Physiol. 43 (2002) 1366–1373.
[66] B. Thangaraj, C.M. Ryan, P. Souda, K. Krause, K.F. Faull, A.P. Weber, P. Fromme, J.P. Whitelegge, Data-directed top-down Fourier-transform mass spectrometry of a large integral membrane protein complex: photosystem II from *Galdieria sulphuraria*, Proteomics 10 (2010) 3644–3656.
[67] H. Fey, D. Piano, R. Horn, D. Fischer, M. Schmidt, S. Ruf, W.P. Schroder, R. Bock, C. Buchel, Isolation of highly active photosystem II core complexes with a His-tagged Cyt b559 subunit from transplastomic tobacco plants, Biochim. Biophys. Acta 1777 (2008) 1501–1509.
[68] Y. Nakamura, T. Kaneko, S. Sato, M. Mimuro, H. Miyashita, T. Tsuchiya, S. Sasamoto, A. Watanabe, K. Kawashima, Y. Kishida, C. Kiyokawa, M. Kohara, M. Matsumoto, A. Matsuno, N. Nakazaki, S. Shimpo, C. Takeuchi, M. Yamada, S. Tabata, Complete genome structure of Gloeobacter violaceus PCC 7421, a cyanobacterium that lacks thylakoids, DNA Res. 10 (2003) 137–145.
[69] J.L. Roose, H.B. Pakrasi, Evidence that D1 processing is required for manganese binding and extrinsic protein assembly into photosystem II, J. Biol. Chem. 279 (2004) 45417–45422.
[70] H. Chen, D. Zhang, J. Guo, H. Wu, M. Jin, Q. Lu, C. Lu, L. Zhang, A Psb27 homologue in *Arabidopsis thaliana* is required for efficient repair of photodamaged photosystem II, Plant Mol. Biol. 61 (2006) 567–575.
[71] M.M. Nowaczyk, R. Hebeler, E. Schlodder, H.E. Meyer, B. Warscheid, M. Rogner, Psb27, a cyanobacterial lipoprotein, is involved in the repair cycle of photosystem II, Plant Cell 18 (2006) 3121–3131.
[72] M. Schubert, U.A. Petersson, B.J. Haas, C. Funk, W.P. Schroder, T. Kieselbach, Proteome map of the chloroplast lumen of *Arabidopsis thaliana*, J. Biol. Chem. 277 (2002) 8354–8365.
[73] J.B. Peltier, O. Emanuelsson, D.E. Kalume, J. Ytterberg, G. Friso, A. Rudella, D.A. Liberles, L. Soderberg, P. Roepstorff, G. von Heijne, K.J. van Wijk, Central functions of the lumenal and peripheral thylakoid proteome of *Arabidopsis* determined by experimentation and genome-wide prediction, Plant Cell 14 (2002) 211–236.
[74] B. Zybailov, H. Rutschow, G. Friso, A. Rudella, O. Emanuelsson, Q. Sun, K.J. van Wijk, Sorting signals, N-terminal modifications and abundance of the chloroplast proteome, PLoS One 3 (2008) e1994.
[75] T. Rajalahti, F. Huang, M.R. Klement, T. Pisareva, M. Edman, M. Sjostrom, A. Wieslander, B. Norling, Proteins in different *Synechocystis* compartments have distinguishing N-terminal features: a combined proteomics and multivariate sequence analysis, J. Proteome Res. 6 (2007) 2420–2434.
[76] K.U. Cormann, J.A. Bangert, M. Ikeuchi, M. Rogner, R. Stoll, M.M. Nowaczyk, Structure of Psb27 in solution: implications for transient binding to photosystem II during biogenesis and repair, Biochemistry 48 (2009) 8768–8770.
[77] K.U. Cormann, M. Ikeuchi, M. Rogner, M.M. Nowaczyk, R. Stoll, Sequence-specific (1)H, (13)C, and (15)N backbone assignment of Psb27 from *Synechocystis* PCC 6803, Biomol. NMR Assign. 3 (2009) 247–249.
[78] P.D. Mabbitt, G.J. Rautureau, C.L. Day, S.M. Wilbanks, J.J. Eaton-Rye, M.G. Hinds, Solution structure of Psb27 from cyanobacterial photosystem II, Biochemistry 48 (2009) 8771–8773.
[79] M.M. Nowaczyk, J. Sander, N. Grasse, K.U. Cormann, D. Rexroth, G. Bernat, M. Rogner, Dynamics of the cyanobacterial photosynthetic network: communication and modification of membrane protein complexes, Eur. J. Cell Biol. 89 (2010) 974–982.
[80] J.L. Roose, H.B. Pakrasi, The Psb27 protein facilitates manganese cluster assembly in photosystem II, J. Biol. Chem. 283 (2008) 4044–4050.
[81] I. Sakurai, N. Mizusawa, S. Ohashi, M. Kobayashi, H. Wada, Effects of the lack of phosphatidylglycerol on the donor side of photosystem II, Plant Physiol. 144 (2007) 1336–1346.
[82] F. Mamedov, M.M. Nowaczyk, A. Thapper, M. Rogner, S. Styring, Functional characterization of monomeric photosystem II core preparations from *Thermosynechococcus elongatus* with or without the Psb27 protein, Biochemistry 46 (2007) 5542–5551.
[83] M.P. Oudot-Le Secq, J. Grimwood, H. Shapiro, E.V. Armbrust, C. Bowler, B.R. Green, Chloroplast genomes of the diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*: comparison with other plastid genomes of the red lineage, Mol. Genet. Genomics 277 (2007) 427–439.
[84] E.V. Armbrust, J.A. Berges, C. Bowler, B.R. Green, D. Martinez, N.H. Putnam, S. Zhou, A.E. Allen, K.E. Apt, M. Bechner, M.A. Brzezinski, B.K. Chaal, A. Chiovitti, A.K. Davis, M.S. Demarest, J.C. Detter, T. Glavina, D. Goodstein, M.Z. Hadi, U. Hellsten, M. Hildebrand, B.D. Jenkins, J. Jurka, V.V. Kapitonov, N. Kroger, W.W. Lau, T.W. Lane, F.W. Larimer, J.C. Lippmeier, S. Lucas, M. Medina, A. Montsant, M. Obornik, M.S. Parker, B. Palenik, G.J. Pazour, P.M. Richardson, T.A. Rynearson, M.A. Saito, D.C. Schwartz, K. Thamatrakoln, K. Valentin, A. Vardi, F.P. Wilkerson, D.S. Rokhsar, The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism, Science 306 (2004) 79–86.
[85] K. Jiroutova, L. Koreny, C. Bowler, M. Obornik, A gene in the process of endosymbiotic transfer, PLoS One 5 (2010) e13234.
[86] Y. Yang, T.A. Ramelot, J.R. Cort, D. Wang, C. Ciccosanti, K. Hamilton, R. Nair, B. Rost, T.B. Acton, R. Xiao, J.K. Everett, G.T. Montelione, M.A. Kennedy, Solution NMR structure of photosystem II reaction center protein Psb28 from *Synechocystis* sp. strain PCC 6803, Proteins 79 (2011) 340–344.
[87] M. Dobakova, R. Sobotka, M. Tichy, J. Komenda, Psb28 protein is involved in the biogenesis of the photosystem II inner antenna CP47 (PsbB) in the cyanobacterium *Synechocystis* sp. PCC 6803, Plant Physiol. 149 (2009) 1076–1086.
[88] D. Yao, T. Kieselbach, J. Komenda, K. Promnares, M.A. Prieto, M. Tichy, W. Vermaas, C. Funk, Localization of the small CAB-like proteins in photosystem II, J. Biol. Chem. 282 (2007) 267–276.
[89] T. Wangdi, S.R. Uppalapati, S. Nagaraj, C.M. Ryu, C.L. Bender, K.S. Mysore, A virus-induced gene silencing screen identifies a role for Thylakoid Formation1 in *Pseudomonas syringae* pv tomato symptom development in tomato and *Arabidopsis*, Plant Physiol. 152 (2010) 281–292.
[90] N. Keren, H. Ohkawa, E.A. Welsh, M. Liberton, H.B. Pakrasi, Psb29, a conserved 22-kD protein, functions in the biogenesis of Photosystem II complexes in *Synechocystis* and *Arabidopsis*, Plant Cell 17 (2005) 2768–2781.
[91] J.E. Froehlich, C.G. Wilkerson, W.K. Ray, R.S. McAndrew, K.W. Osteryoung, D.A. Gage, B.S. Phinney, Proteomic study of the *Arabidopsis thaliana* chloroplastic envelope membrane utilizing alternatives to traditional two-dimensional electrophoresis, J. Proteome Res. 2 (2003) 413–425.
[92] T. Kleffmann, D. Russenberger, A. von Zychlinski, W. Christopher, K. Sjolander, W. Gruissem, S. Baginsky, The *Arabidopsis thaliana* chloroplast proteome reveals pathway abundance and novel protein functions, Curr. Biol. 14 (2004) 354–362.
[93] J.B. Peltier, A.J. Ytterberg, Q. Sun, K.J. van Wijk, New functions of the thylakoid membrane proteome of *Arabidopsis thaliana* revealed by a simple, fast, and versatile fractionation strategy, J. Biol. Chem. 279 (2004) 49367–49383.
[94] J.B. Peltier, Y. Cai, Q. Sun, V. Zabrouskov, L. Giacomelli, A. Rudella, A.J. Ytterberg, H. Rutschow, K.J. van Wijk, The oligomeric stromal proteome of *Arabidopsis thaliana* chloroplasts, Mol. Cell. Proteomics 5 (2006) 114–133.
[95] Q. Wang, R.W. Sullivan, A. Kight, R.L. Henry, J. Huang, A.M. Jones, K.L. Korth, Deletion of the chloroplast-localized Thylakoid formation1 gene product in *Arabidopsis* leads to deficient thylakoid formation and variegated leaves, Plant Physiol. 136 (2004) 3594–3604.
[96] J. Huang, J.P. Taylor, J.G. Chen, J.F. Uhrig, D.J. Schnell, T. Nakagawa, K.L. Korth, A.M. Jones, The plastid protein THYLAKOID FORMATION1 and the plasma membrane G-protein GPA1 interact in a novel sugar-signaling mechanism in *Arabidopsis*, Plant Cell 18 (2006) 1226–1238.
[97] L. Zhang, Q. Wei, W. Wu, Y. Cheng, G. Hu, F. Hu, Y. Sun, Y. Zhu, W. Sakamoto, J. Huang, Activation of the heterotrimeric G protein alpha-subunit GPA1 suppresses the ftsh-mediated inhibition of chloroplast development in *Arabidopsis*, Plant J. 58 (2009) 1041–1053.
[98] M. Lindahl, C. Spetea, T. Hundal, A.B. Oppenheim, Z. Adam, B. Andersson, The thylakoid FtsH protease plays a role in the light-induced turnover of the photosystem II D1 protein, Plant Cell 12 (2000) 419–431.
[99] W. Sakamoto, A. Zaltsman, Z. Adam, Y. Takahashi, Coordinated regulation and complex formation of yellow variegated1 and yellow variegated2, chloroplastic FtsH metalloproteases involved in the repair cycle of photosystem II in *Arabidopsis* thylakoid membranes, Plant Cell 15 (2003) 2843–2855.

[100] A. Zaltsman, N. Ori, Z. Adam, Two types of FtsH protease subunits are required for chloroplast biogenesis and Photosystem II repair in *Arabidopsis*, Plant Cell 17 (2005) 2782–2790.
[101] S. Bailey, E. Thompson, P.J. Nixon, P. Horton, C.W. Mullineaux, C. Robinson, N.H. Mann, A critical role for the Var2 FtsH homologue of *Arabidopsis thaliana* in the photosystem II repair cycle in vivo, J. Biol. Chem. 277 (2002) 2006–2011.
[102] Q. Wei, W. Zhou, G. Hu, J. Wei, H. Yang, J. Huang, Heterotrimeric G-protein is involved in phytochrome A-mediated cell death of *Arabidopsis* hypocotyls, Cell Res. 18 (2008) 949–960.
[103] Y. Uno, M.A. Rodriguez Milla, E. Maher, J.C. Cushman, Identification of proteins that interact with catalytically active calcium-dependent protein kinases from *Arabidopsis*, Mol. Genet. Genomics 281 (2009) 375–390.
[104] S.Y. Zhu, X.C. Yu, X.J. Wang, R. Zhao, Y. Li, R.C. Fan, Y. Shang, S.Y. Du, X.F. Wang, F.Q. Wu, Y.H. Xu, X.Y. Zhang, D.P. Zhang, Two calcium-dependent protein kinases, CPK4 and CPK11, regulate abscisic acid signal transduction in *Arabidopsis*, Plant Cell 19 (2007) 3019–3036.
[105] T. Wangdi, S.R. Uppalapati, S. Nagaraj, C.M. Ryu, C.L. Bender, K.S. Mysore, A role for chloroplast-localized Thylakoid Formation 1 (THF1) in bacterial speck disease development, Plant Signal. Behav. 5 (2010) 425–427.
[106] Y. Kashino, T. Takahashi, N. Inoue-Kashino, A. Ban, Y. Ikeda, K. Satoh, M. Sugiura, Ycf12 is a core subunit in the photosystem II complex, Biochim. Biophys. Acta 1767 (2007) 1269–1275.
[107] N. Inoue-Kashino, T. Takahashi, A. Ban, M. Sugiura, Y. Takahashi, K. Satoh, Y. Kashino, Evidence for a stable association of Psb30 (Ycf12) with photosystem II core complex in the cyanobacterium *Synechocystis* sp. PCC 6803, Photosynth. Res. 98 (2008) 323–335.
[108] V.V. Goremykin, K.I. Hirsch-Ernst, S. Wolfl, F.H. Hellwig, Analysis of the *Amborella trichopoda* chloroplast genome sequence suggests that amborella is not a basal angiosperm, Mol. Biol. Evol. 20 (2003) 1499–1505.
[109] M. Sugiura, S. Harada, T. Manabe, H. Hayashi, Y. Kashino, A. Boussac, Psb30 contributes to structurally stabilise the Photosystem II complex in the thermophilic cyanobacterium *Thermosynechococcus elongatus*, Biochim. Biophys. Acta 1797 (2010) 1546–1554.
[110] P. Faller, C. Fufezan, A. Rutherford (Eds.), Side-path Electron Donors: Cytochrome b559, Chlorophyll Z and β-Carotene, "Photosystem II" Advances in Photosynthesis and Respiration, vol. 22, Springer, 2005, pp. 347–365.
[111] D.H. Stewart, G.W. Brudvig, Cytochrome b559 of photosystem II, Biochim. Biophys. Acta 1367 (1998) 63–87.
[112] A. Guskov, A. Gabdulkhakov, M. Broser, C. Glockner, J. Hellmich, J. Kern, J. Frank, F. Muh, W. Saenger, A. Zouni, Recent progress in the crystallographic studies of photosystem II, Chemphyschem 11 (2010) 1160–1171.
[113] R. Nagao, A. Ishii, O. Tada, T. Suzuki, N. Dohmae, A. Okumura, M. Iwai, T. Takahashi, Y. Kashino, I. Enami, Isolation and characterization of oxygen-evolving thylakoid membranes and photosystem II particles from a marine diatom *Chaetoceros gracilis*, Biochim. Biophys. Acta 1767 (2007) 1353–1362.
[114] A. Okumura, R. Nagao, T. Suzuki, S. Yamagoe, M. Iwai, K. Nakazato, I. Enami, A novel protein in Photosystem II of a diatom *Chaetoceros gracilis* is one of the extrinsic proteins located on lumenal side and directly associates with PSII core components, Biochim. Biophys. Acta 1777 (2008) 1545–1551.
[115] R. Nagao, T. Tomo, E. Noguchi, S. Nakajima, T. Suzuki, A. Okumura, Y. Kashino, M. Mimuro, M. Ikeuchi, I. Enami, Purification and characterization of a stable oxygen-evolving Photosystem II complex from a marine centric diatom, *Chaetoceros gracilis*, Biochim. Biophys. Acta 1797 (2010) 160–166.
[116] I. Enami, S. Kikuchi, T. Fukuda, H. Ohta, J.R. Shen, Binding and functional properties of four extrinsic proteins of photosystem II from a red alga, *Cyanidium caldarium*, as studied by release-reconstitution experiments, Biochemistry 37 (1998) 2787–2793.
[117] I. Enami, H. Murayama, H. Ohta, M. Kamo, K. Nakazato, J.R. Shen, Isolation and characterization of a Photosystem II complex from the red alga *Cyanidium caldarium*: association of cytochrome c-550 and a 12 kDa protein with the complex, Biochim. Biophys. Acta 1232 (1995) 208–216.
[118] R. Nagao, A. Moriguchi, T. Tomo, A. Niikura, S. Nakajima, T. Suzuki, A. Okumura, M. Iwai, J.R. Shen, M. Ikeuchi, I. Enami, Binding and functional properties of five extrinsic proteins in oxygen-evolving photosystem II from a marine centric diatom, *Chaetoceros gracilis*, J. Biol. Chem. 285 (2010) 29191–29199.
[119] K.M. Wegener, S. Bennewitz, R. Oelmuller, H.B. Pakrasi, The Psb32 protein aids in repairing photodamaged photosystem II in the *Cyanobacterium synechocystis* 6803, Mol. Plant (2011), doi:10.1093/mp/ssr044.
[120] S. Sirpio, Y. Allahverdiyeva, M. Suorsa, V. Paakkarinen, J. Vainonen, N. Battchikova, E.M. Aro, TLP18.3, a novel thylakoid lumen protein regulating photosystem II repair cycle, Biochem. J. 406 (2007) 415–425.
[121] P. Mulo, S. Sirpio, M. Suorsa, E.M. Aro, Auxiliary proteins involved in the assembly and sustenance of photosystem II, Photosynth. Res. 98 (2008) 489–501.
[122] N.A. Dolganov, D. Bhaya, A.R. Grossman, Cyanobacterial protein with similarity to the chlorophyll a/b binding proteins of higher plants: evolution and regulation, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 636–640.
[123] Q. He, N. Dolganov, O. Bjorkman, A.R. Grossman, The high light-inducible polypeptides in *Synechocystis* PCC6803. Expression and function in high light, J. Biol. Chem. 276 (2001) 306–314.
[124] K. Mikami, Y. Kanesaki, I. Suzuki, N. Murata, The histidine kinase Hik33 perceives osmotic stress and cold stress in *Synechocystis* sp PCC 6803, Mol. Microbiol. 46 (2002) 905–915.
[125] C. Funk, W. Vermaas, A cyanobacterial gene family coding for single-helix proteins resembling part of the light-harvesting proteins from higher plants, Biochemistry 38 (1999) 9397–9404.
[126] B.R. Green, W. Kühlbrandt, Sequence conservation of light-harvesting and stress-response proteins in relation to the three-dimensional molecular structure of LHCII, Photosynth. Res. 44 (2002) 139–148.
[127] J.A. Neilson, D.G. Durnford, Structural and functional diversification of the light-harvesting complexes in photosynthetic eukaryotes, Photosynth. Res. 106 (2010) 57–71.
[128] D. Bhaya, A. Dufresne, D. Vaulot, A. Grossman, Analysis of the hli gene family in marine and freshwater cyanobacteria, FEMS Microbiol. Lett. 215 (2002) 209–219.
[129] S. Bailey, M.R. Clokie, A. Millard, N.H. Mann, Cyanophage infection and photoinhibition in marine cyanobacteria, Res. Microbiol. 155 (2004) 720–725.
[130] D. Lindell, M.B. Sullivan, Z.I. Johnson, A.C. Tolonen, F. Rohwer, S.W. Chisholm, Transfer of photosynthesis genes to and from *Prochlorococcus* viruses, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 11013–11018.
[131] M. Havaux, G. Guedeney, Q. He, A.R. Grossman, Elimination of high-light-inducible polypeptides related to eukaryotic chlorophyll a/b-binding proteins results in aberrant photoacclimation in *Synechocystis* PCC6803, Biochim. Biophys. Acta 1557 (2003) 21–33.
[132] H. Xu, D. Vavilin, C. Funk, W. Vermaas, Multiple deletions of small Cab-like proteins in the cyanobacterium *Synechocystis* sp. PCC 6803: consequences for pigment biosynthesis and accumulation, J. Biol. Chem. 279 (2004) 27971–27979.
[133] D. Kirilovsky, Photoprotection in cyanobacteria: the orange carotenoid protein (OCP)-related non-photochemical-quenching mechanism, Photosynth. Res. 93 (2007) 7–16.
[134] P. Storm, M.A. Hernandez-Prieto, L.L. Eggink, J.K. Hoober, C. Funk, The small CAB-like proteins of *Synechocystis* sp. PCC 6803 bind chlorophyll. In vitro pigment reconstitution studies on one-helix light-harvesting-like proteins, Photosynth. Res. 98 (2008) 479–488.
[135] H. Xu, D. Vavilin, C. Funk, W. Vermaas, Small Cab-like proteins regulating tetrapyrrole biosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803, Plant Mol. Biol. 49 (2002) 149–160.
[136] D. Vavilin, D. Yao, W. Vermaas, Small Cab-like proteins retard degradation of photosystem II-associated chlorophyll in *Synechocystis* sp. PCC 6803: kinetic analysis of pigment labeling with 15N and 13C, J. Biol. Chem. 282 (2007) 37660–37668.
[137] Q. Wang, S. Jantaro, B. Lu, W. Majeed, M. Bailey, Q. He, The high light-inducible polypeptides stabilize trimeric photosystem I complex under high light conditions in *Synechocystis* PCC 6803, Plant Physiol. 147 (2008) 1239–1250.
[138] S. Jansson, A protein family saga: from photoprotection to light-harvesting (and back?), in: B. Demmig-Adams (Ed.), Photoprotection, Photoinhibition, GeneRegulation and Environment, Springer, The Netherlands, 2005, pp. 145–153.
[139] R. Sobotka, S. McLean, M. Zuberova, C.N. Hunter, M. Tichy, The C-terminal extension of ferrochelatase is critical for enzyme activity and for functioning of the tetrapyrrole pathway in *Synechocystis* strain PCC 6803, J. Bacteriol. 190 (2008) 2086–2095.
[140] K. Promnares, J. Komenda, L. Bumba, J. Nebesarova, F. Vacha, M. Tichy, Cyanobacterial small chlorophyll-binding protein ScpD (HliB) is located on the periphery of photosystem II in the vicinity of PsbH and CP47 subunits, J. Biol. Chem. 281 (2006) 32705–32713.
[141] G. Kufryk, M.A. Hernandez-Prieto, T. Kieselbach, H. Miranda, W. Vermaas, C. Funk, Association of small CAB-like proteins (SCPs) of *Synechocystis* sp. PCC 6803 with Photosystem II, Photosynth. Res. 95 (2008) 135–145.
[142] J. Komenda, M. Tichy, L.A. Eichacker, The PsbH protein is associated with the inner antenna CP47 and facilitates D1 processing and incorporation into PSII in the cyanobacterium *Synechocystis* PCC 6803, Plant Cell Physiol. 46 (2005) 1477–1483.
[143] M. Boehm, E. Romero, V. Reisinger, J. Yu, J. Komenda, L.A. Eichacker, J.P. Dekker, P.J. Nixon, Investigating the early stages of photosystem II assembly in *Synechocystis* sp. PCC 6803: isolation of CP47 and CP43 complexes, J. Biol. Chem. 286 (2011) 14812–14819.
[144] U. Andersson, M. Heddad, I. Adamska, Light stress-induced one-helix protein of the chlorophyll a/b-binding family associated with photosystem I, Plant Physiol. 132 (2003) 811–820.
[145] M.A. Hernandez-Prieto, T. Tibiletti, L. Abasova, D. Kirilovsky, I. Vass, C. Funk, The small CAB-like proteins of the cyanobacterium *Synechocystis* sp. PCC 6803: their involvement in chlorophyll biogenesis for Photosystem II, Biochim. Biophys. Acta 1907 (2011) 1143–1151.
[146] D. Yao, T. Kieselbach, J. Komenda, K. Promnares, M. Hernandez-Prieto, M. Tichy, W. Vermaas, C. Funk, Localization of the small CAB-like proteins in the photosynthetic complexes, J. Biol. Chem 282 (2007) 267–276.